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GRANT NUMBER DAMD17-98-1-8002

TITLE: Disaster Relief and Emergency Medical Services Project (DREAMS TM): Clinical and Basic Science Projects

PRINCIPAL INVESTIGATOR: Ward Casscells, M.D.

CONTRACTING ORGANIZATION: The University of Texas
Houston, Texas 77225-0708

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13. ABSTRACT *(Maximum 200 words)*

DREAMS clinical and basic science projects complement the digital EMS effort by investigating the mechanisms of tissue injury in order to minimize the mortality and mortality of trauma and "natural" injuries such as heart attacks. We have a broad effort aimed at the major problems:oxidation(common to resuscitation/reperfusion injuries of heart, brain ,liver and kidney),toxins,apoptosis,inflammation, cell proliferation, and angiogenesis.

We have made progress in understanding the genes controlling anthrax, and those of the human P450 defense system;we have developed new probes to help interventionalists and surgeons diagnose tissue types and distinguish inflammation and necrosis in real time; a field -implantable pump has been developed to boost cardiac output in order to prevent the viscious cycle of low cardiac output causing ischemia; we have increased our understanding of the the denitration of NO(the gas that regulates blood pressure, clotting, oxidant defense against microbes, nerve cell communication, cell proliferation and death); we have learned more about the roles played by PPAR gamma, ICAM1, E-selectin and VCAM1 in inflammation; finally, we have learned new ways to introduce genes into cells and have demonstrated new possibilities in gene therapy while uncovering new problems with a current technique of gene therapy.

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FOREWORD

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PI - Signature Date

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DREAMS ANNUAL REPORT – 1999

Project 1C. Automatic External Defibrillators

During Fiscal Year 1999, we made considerable progress in persuading industries to purchase and deploy automatic external defibrillators. During the previous year, Drs. Duke, Casscells, and Galloway had made a video to educate the small-business owners, civic leaders, and medical directors and safety directors at large corporations. Together with the American Heart Association volunteers, Dr. Casscells and the City of Houston Fire Department's EMS Director, David Perrse, M.D., made numerous presentations in person and on radio and television about the potential of these defibrillators to save lives in community settings. Of particular importance were two events in 1999: first, the reporting of lives saved by the use of these defibrillators; and second, the passage of a bill in the State Legislature, which was subsequently signed by Governor Bush, to limit the legal liability of those who purchase, maintain and use the automatic external defibrillators. Testimony by Drs. Casscells and Duke was featured in the House and Senate hearings.

Dozens of Houston industries have now purchased defibrillators, as have many churches, civic groups and many clubs and, in some cases, individuals. This project has now germinated and has been "adopted" by the City's Fire Department, particularly gratifying is the recent decision by the city and the AHA to launch a program to promote public access defibrillation (and not just place AED's where EMT's, RN's and MD's can find them). The DREAMS principal investigators now feel that the leadership by the City and the American Heart Association (AHA) will be sufficient to sustain this program and no further DREAMS funds will be budgeted, thereby successfully terminating Project 1C.

Project 1F. Fibroblast Growth Factor (FGF2) to Enhance Angiogenesis and Healing

This project encountered several difficulties this year. The small amount of DREAMS funds budgeted required supplementary funds to determine whether FGF has a real impact in angiogenesis and wound healing in animals and patients. DREAMS funds were thus used for salary support to coordinate and write up some of this work. The animal studies showed that FGF has a hypotensive

effect, which limited its utility in an *in vivo* treatment. Even more important was the fact that in a small Phase I clinical trial (in which DREAMS P.I. Ward Casscells, M.D., participated, with DREAMS salary support for himself but no DREAMS funds for the research), three patients developed severe proteinuria, and in one patient this was irreversible. Finally, early results from clinical trials conducted by others (with no participation by Dr. Casscells) suggest that FGF's angiogenic effect in humans will be small or nonexistent. Therefore, this project will be concluded. The attached manuscript has been submitted for publication.

Project 3M. Electrical Impedance and Near Infrared...

This project began with the goal of comparing electrical impedance testing with near-infrared spectroscopic analysis for diagnosis of vulnerable atherosclerotic plaque. We subsequently dropped the electrical impedance aspect because: 1) We realized the near-IR approach could not only convey information about the presence of lipid (the major utility of electrical impedance for plaque analysis) and could also confer information relevant to the diagnosis of inflammation generally and so be of broader utility than just for atherosclerosis (i.e., including wounds, burns, toxic exposures, autoimmune conditions and cancers); 2) We found that the electrical impedance idea was not novel and indeed had been patented several years ago, with no subsequent published research in that area, suggesting technical unfeasibility.

In contrast, near-IR work has progressed well. The attached abstract by Wang, Guo, Klima, Willerson and Casscells indicates that near-infrared spectral differences were found between various types of living atherosclerotic plaques. Subsequent research has found differences between three wave lengths in contrast to the single band identified by Robert Lodder and James Muller, who studied formal in fixed sections.

We reasoned that something unique to the living atherosclerotic plaque might be involved in the differences between our work and theirs. We hypothesized that, in addition to fat and the oxidative reactions of activated inflammatory cells, plaques--and wounds and abscesses in general--would also exhibit hypoxia, glucopenia and acidosis. All three factors have been shown to affect NIR spectra

in other systems.

We therefore examined the hypothesis that atherosclerotic plaques are heterogeneous with respect of pH. As described in the attached abstract by R. John and colleagues, we found marked pH heterogeneity in atherosclerotic plaque, both in living human specimens from carotid endarterectomy procedures, studied immediately *ex vivo*, and living specimens of human umbilical arteries obtained after being discarded by the pathologist in the delivery room. Watanabe atherosclerotic rabbits also exhibited pH netcrogenity.

The major findings were that the average pH was higher in plaques than in Watanabe rabbits, which in turn were higher than those of human umbilical arteries. Acidic regions were localized to the yellow (lipid-rich) regions of the plaque, whereas the calcified areas had a high pH (presumably due to the calcium carbonate. The lack of calcification in the Watanabe rabbit lesions is consistent with their generally lower pH.

This is a novel finding and raises major questions as to the cause of the pH heterogeneity and its effects on plaque functions, including gene expression, enzyme activity (particularly metabolic and matrix-degrading enzymes) and other areas such as release of growth factors from matrix-binding sites.

Plans over the next year include: 1) Increasing the numbers of specimens analyzed to increase the statistical strength of the subgroup analyses. 2) Determining the role of the protein-lipid ratio in the NIR characteristic spectral signature. 3) Determining whether the experimental pH variation can be detected by NIR spectroscopy. We anticipate that in the coming year at least two manuscripts will be published. Later in the year, we hope to compare temperature measurements vs. NIR measurements for their accuracy in the detection of the vulnerable plaque.

Also, we will be exploring the possible utility of NIR in the characterization of wounds with regard to the presence of infection, wound healing rates, presence of foreign bodies, vascularity, viability and necrosis.

Project 2A.

Project 2A initially had the goal of using intravenous injections of FGF-2 to stimulate angiogenesis. This approach has not proven efficacious in our hands. Therefore, we focused our attention and funds on Project 1F, in which we attempted to use FGF-2 to encourage endothelial regrowth on intravascular prostheses and stents. Working with Tim Scott-Burden, Ph.D., we found better efficacy with a vascular endothelial growth-factor transgenic approach, and for this reason Projects 2A and 1F were terminated, with the conclusion that, while an angiogenesis effect of FGF-2 had not been excluded or disproved, the effect was not large enough, in these models at least, to justify further work.

Project 2C. Infrared Detection of Inflammation.

This project made gratifying progress in FY 1999. Its goal is to develop new devices for the detection of inflammation to assist in the diagnosis and prognosis of atherosclerotic plaques, cancers, wounds and a variety of autoimmune diseases. Also, in some cases, the thermal approach will have therapeutic potential as an anti-inflammatory treatment.

Work to date has focused on the detection of inflamed atherosclerotic plaques because of their ready availability and because heart attack and stroke together account for more than half of the deaths in the United States and by 2010 will be the leading causes of death in the world.

Imaging atherosclerotic plaques to determine if heat will prove to be the best way of predicting plaques at risk of precipitating heart attack and stroke is being undertaken by three methods. The first is infrared fiberoptic imaging. We bundled together arrays of chalcogenide fibers into a catheter capable of 100 micron spatial resolution and 0.1° C. thermal resolution. With this catheter, we have been able to image temperature heterogeneity in plaques *ex vivo* and *in vivo*. This catheter has limited flexibility and the fibers are fragile. In addition, improved spatial resolution would be desirable to give a less grainy image. Plans for improved prototypes are described below. (See

attached abstract by Guo and colleagues.)

The second approach has been the construction of a catheter which, when advanced out of its sheath, springs open to reveal four nitinol-shaped memory wires, each of which is equipped with a 0.003-inch thermocouple. The catheter has a temperature resolution of 0.005° C. and spatial resolution of 0.5 mm., with an acquisition time of 0.01 second. The problems with this catheter include the difficulty in shielding the thermocouple's measurement of wall temperature from the temperature of the blood and the limited number of data points, and the fact that it has to contact the vessel wall and then be "deflated" before it is moved to the next spot (increasing the duration of the study). Future prototypes will compare thermocouples to thermistors, will use more sensors and will insulate these sensors better. The basket arrangement allows blood to flow down the vessel during the period of measurement, such that no proximal occluding balloon or perfusion lumen is needed, which are two advantages to the infrared approach. (See attached abstract by Naghavi and colleagues).

The third approach consists of simple infrared camera imaging of human AV grafts. Patients with kidney failure typically have a graft placed just under the skin of the forearm for arterial and venous access for purposes of hemodialysis. Most of these patients have very little subcutaneous tissue, so that inflammation in the graft is detectable by simply palpating the arms of these patients. These areas are frequently swollen and tender. The redness of inflammation does not project through the skin, but the temperature does. We have been able to image hot atherosclerotic plaques in these patients with an external camera. No contact with the skin is required. This technique (described in the attached abstract by Gul and colleagues) will enable us to determine the natural history of the hot plaque by following these patients prospectively.

Work of FY 2000 will focus on: 1) The above-described longitudinal study to determine whether the hot plaque indeed goes on to occlude the vessel. 2) Making the above-described improvement in the nitinol-shaped memory basket catheter. 3) Improving the infrared catheter by utilizing smaller (25-micron) fibers, now available from Raytheon, and equipping the distal tip with a cone-shaped aluminum mirror to create a side-looking catheter. The thermistor/thermocouple and infrared approaches will then be compared. Late in the year we will be investigating the potential of

bolometer ehip-based systems, which may prove competitive with the infrared fiber approach to thermal imaging.

Therapeutic Heating

In the first year of the DREAMS project, we described the observation that heating in the high-fever range (41° C.) could paradoxically cause inflamed tissues to cool off . On histologic examination of these human carotid endarterectomy specimens (which were heated immediately after removal from the patient for 15 minutes and then returned to a 37° incubator for several hours prior to remeasurement of temperature and then fixation), we observed that the macrophages were much more prone to thermal apoptosis than the endothelial and smooth-muscle cells in the plaque.

Over the past year, we extended these observations to begin to explore the molecular mechanisms involved. In the attached abstract by Lal and colleagues, we describe a down-regulation of tumor necrosis factor alpha and interleukin-6, two pro-inflammatory cytokines, when human carotid endarterectomy specimens were heated at 42° C. for 15 minutes, followed by six hours at 37°.

In the coming year, we will extend these immunocytochemical observations and look at the protein abundance by western blotting and at the RNA levels by *in situ* hybridization and semiquantitative RT-PCR. Also, we will be examining the effect of heating on NF k B levels.

We next looked at the effect of physiologic heating *in vivo*. Watanabe hypercholesterolemia rabbits were fed a cholesterol-rich diet for six months to increase the number of atherosclerotic-like lesions in their aortas. We then heated the atherosclerotic tissues, using a thermal ablation catheter, to 42° C. for 15 minutes. A significant and selective increase in macrophage apoptosis was noted in the intimal layer by TUNEL histologic analysis. Now the optimal temperature and duration of heating will need to be determined.

We have extended these observations still further by asking whether heat could be used to prevent or inhibit restenosis. Robert Schwartz and colleagues at Mayo reported that heating can inhibit

smooth-muscle proliferation. Because Pedro Moreno and others at MGH have identified macrophage content as a key marker of risk of restenosis, we reasoned that selective induction of apoptosis in macrophages, by gentle heating, might also inhibit restenosis. Because a single treatment might not be sufficient, we sought a noninvasive way of promoting this heating. We are exploring radiofrequency heating of ferro magnetic stents and ultrasound heating of polymeric stents. As described by Naghavi et al in the attached abstract, phantom experiments conducted with muscle specimens from the supermarket demonstrated that, with the right ultrasound intensity and frequency, some stent materials (for example, some PVC polymers) heat faster than the surrounding tissue. Interestingly, other polymers, such as PTFE, Teflon, Lexan and certain types of PVC, did not show this selective heating. These studies suggest that external heating could be used to rid intravascular stents and other polymeric implants of excessive inflammatory cells. In the case of arterial stents, this could prove to be an effective and nonradioactive means of preventing apoptosis. Studies in the coming year will be conducted *in vivo* in our animal models, and further polymer/ultrasound permutations will be explored in phantom studies on the bench top. Overall, these studies show considerable progress in the use of thermal imaging to identify disease and perhaps predict its course and, for diseases characterized by excess inflammation, to decrease the amount of inflammation and possibly improve the course of the disease.

ANNUAL PROGRESS REPORT

Project Title:	**"Disaster Relief and Emergency Medical Services Project (DREAMS): Clinical and Basic Science Projects"**
Awarded to:	**The University of Texas-Houston Health Science Center**
Principal Investigator:	**Ward Casscells, M.D.**
Grant No.:	**DAMD17-98-1-8002**
Reporting Period:	**11/01/98 to 10/31/99**

Subproject Number and Title: **"Initial Evaluation of a New Axial Flow Pump, Inserted by Minimally Invasive Thoracotomy, to Maintain Cardiac Output in a Porcine Model of Cardiogenic and Hemorrhagic Shock" (Subproject No. 2-D)**

Subproject Investigator Signature: _____
O.H. Frazier, M.D.

Project Objectives:

The purpose of this project is to investigate a novel approach to reducing mortality and morbidity due to injuries suffered by military personnel in combat zones. Specifically, the objective of this research is to evaluate the use of an implantable mechanical cardiac assist device, in conjunction with standard volume and/or blood replacement, for treatment of hemorrhagic shock resulting from injuries sustained in the combat setting.

The specific aims of this project are:

1. Refine the animal model of hemorrhagic shock in pigs.
2. Demonstrate decreased mortality in the animal model through application of circulatory support using a newly developed cardiac assist pump.
3. Demonstrate that this cardiac assist pump is lightweight, portable, easily implanted and practical to use on the battlefield.

Progress Summary:

A total of fifteen studies have been performed as of this reporting period. Eight were performed as controls; the animals were hemorrhaged and resuscitated with fluid administration (2cc LRS/ 1cc blood loss). Seven studies were randomized for resuscitation and a LVAD implant. A commercially available centrifugal flow pump was selected for the test group. One animal in the LVAD group was excluded due to myocardial fibrillation prior to hemorrhaging (see chart). The remaining LVAD animals were hemorrhaged, resuscitated, and underwent a LVAD implantation. Two studies (13-A, 14-B) were performed to determine the feasibility of the bovine as an animal model for future studies. Data from these studies were used as the basis for discussion and evaluation of the hemorrhagic shock model. Upon completion of the two studies, several key decisions were made regarding the surgery and instrumentation of the bovine animal model.

Surgery Date	Study No. C/P*	Animal No.	Animal Wt. (kg)	Survival Time **	Amount of Hemorrhage (ml)	Volume administered (liters)	Total U/O
09/14/98	1-A	P-585	51	1.5	400	2.8	400
10/13/98	2-A	P-593	56	8.0	1000	9.5	1000
10/26/98	3-A	P-594	56	18.0	1580	19.2	4148
11/16/98	4-B	P-595	60	7.5	750	7.0	1450
12/09/98	5-B	P-597	53	4.0	680	7.7	350
12/15/98	6-A	P-598	60	15.0	945	22.6	4060
01/04/99	7-A	P-602	71	8.0	1475	12.0	1400
03/16/99	8-B	P-604	50	17	900	18.8	1500
03/29/99	9-A	P-633	56	11	450	9.2	950
05/12/99	10-B	P-635	57	21	463	13.8	3950
05/19/99[∂]	11-B	P-637	49	0	0	0	0
05/26/99	12-B	P-638	52	5	702	10.5	1500
08/09/99	13-A	B-1187	98	5.5	346	6.0	2000
09/07/99	14-B	B-1200	98	7.5	594	34.3	5900
09/20/99	15-A	P-677	40	1.5	1335	7.9	1800

*A=Control and B =LVAD

**Begins at end of Hemorrhage (Hrs.)

[∂]This animal was not included in our data

Texas Heart Institute's Associate Investigator, Dr. Branislav Radovancevic, Dr. Harald Eichstaedt, surgical research fellow, and Project Manager Mr. Dan Tamez traveled separately or together to San Antonio a total of two times in this reporting period. The THI team participated in animal studies of the parallel program at Brooke Army Medical Center (BAMC). Additionally, the THI group met with BAMC investigators to finalize the strategic plan and protocol for the Phase II studies at THI. *(Note: As stated previously, the DREAMS program does not fund the work at BAMC. BAMC investigators, through separate funding, have pioneered the development of the hemorrhagic shock model in the pig. The approved DREAMS workscope includes travel to San Antonio to learn from the experience at BAMC, in order to minimize redundancy and reduce costs).*

Planned Work

A new protocol has been submitted by the investigator to the Texas Heart Institute's Institutional Animal Care and Use Committee. The committee approved the animal research protocol and the use of 20 animals. This protocol will continue our earlier work and allows us to investigate the use of the larger bovine animal model. The protocol is for 20 animals subdivided into two groups.

The new Phase II project will allow the completion of the work begun earlier at the Texas Heart Institute (during "Phase I DREAMS funding, THI IACUC Protocol 98-01). We will perform twenty studies, 10 studies using the bovine animal model and 10 with the porcine. The funds remaining from the Phase I DREAMS award will be sufficient to complete 10 studies. The new funding will be sufficient for the remaining 10 experiments (see chart, below).

DREAMS Studies to be conducted:

	1999												2000																								
Funding Source:	OCT.				NOV.				DEC.				JAN.				FEB.				MAR.				APR.				MAY				JUNE				JULY				AUG.				SEPT.				TOTAL
Phase I									X			X	X			X	X			X	X			X	X																						10
Phase II																												X			X			X			X			X			X			X	10

QUARTERLY PROGRESS REPORT file: Drm99oct.Rpt

PROJECT TITLE: "Disaster Relief and Emergence Medical Services Project (Dreams): Clinical and Basic Science Projects"
THE UNIVERSITY OF TEXAS - HOUSTON HEALTH SCIENCE CENTER
PRINCIPAL INVESTIGATOR: Ward Casscells, M.D.
GRANT NO: DAMD19-98-1-8002
REPORTING PERIOD: Aug 1 - Oct 31, 1999

Subproject Number/Title: II. Basic Science Project E: "Nitric oxide synthase inhibition therapy to prevent multi-organ system failure after hemorrhagic shock"

Investigator Name: **RF Lodato**

Investigator Signature: RF Lodato

Progress Report:
Describe scientific progress for the quarter in terms of the tasks or objectives listed in the statement of work for this project. Explain deviations where this is not possible. Include data where possible. Present a brief statement of plans or milestones for the next quarter. *Do not exceed two pages.*

During this quarter, Abbas Mirza, M.D., Research Associate, has been trained in cardiovascular surgery in animals to implant chronic aortic blood flow probes and vascular access catheters. Recently, he has successfully obtained high fidelity 10 MHz Doppler aortic blood flow signals, as shown in Figure 1. These chronically implanted flow probes allow long term and continuous cardiac output measurements from a conscious animal.

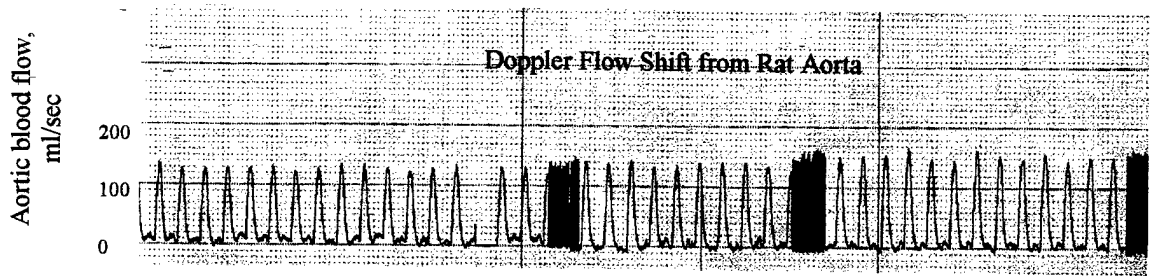

Fig 1

Plans for next quarter: We will develop software to retrieve, store and analyze the hemodynamic data from the animals, purchase the needed pharmacological agents, and acquire and develop micro-infusion systems for continuous intravenous drug delivery.

ANNUAL PROGRESS REPORT

PROJECT TITLE: "Disaster Relief and Emergency Medical Services Project (DREAMS): Clinical and Basic Science Projects
THE UNIVERSITY OF TEXAS - HOUSTON HEALTH SCIENCE CENTER
PRINCIPAL INVESTIGATOR: Ward CASSCELLS, M.D.
GRANT NO: DAMD17-98-1-8002
REPORTING PERIOD: 11/1/98-10/31/99

Subproject Number/Title:
IIIA. Role of sentrin in apoptosis

Investigator Name:
Edward T.H. Yeh, M.D.

Investigator Signature:

Progress Report: Describe scientific progress for the quarter in terms of the tasks or objectives listed in the statement of work for this project. Explain deviations where this is not possible. Include data where possible. Present a brief statement of plans or milestones for the next quarter. *Do not exceed two pages.*

Apoptosis, programmed cell death, plays an important role in reperfusion injury and in cell death caused by radiation, trauma and hypoxia. We have discovered a novel ubiquitin-like protein called sentrin-1 which interacts with the cell death domains of Fas and tumor necrosis factor receptor-1. Over-expression of sentrin-1 in cells can inhibit anti-Fas and tumor necrosis factor induced cell death.

In order to test the effect of sentrin-1 over-expression in medically relevant cells, and eventually in animal models, we have begun to generate recombinant adenovirus ca recombinant adenovirus that expresses HA-tagged sentrin-1. The presence of HA-sentrin 1 in the viral genome was verified by performing a Western blot analysis of the crude cell extract from HUVECs infected with the virus. Briefly, one day after infecting HUVEC with recombinant viruses carrying either HA-sentrin-1 or β-Gal, the cells were lysed and processed for Western

blot analysis as described previously (20). As shown in **Fig. 1**, sentrin-1 monomers and sentrin-1 conjugates were only detected in HUVEC infected with recombinant HA-sentrin-1 virus (lane 2).

In this budget period, we have prepared recombinant virus expressing sentrin proteins with Gly residues involved in conjugation deleted. We are in the process of purifying a large amount of the recombinant virus to be used for functional studies.

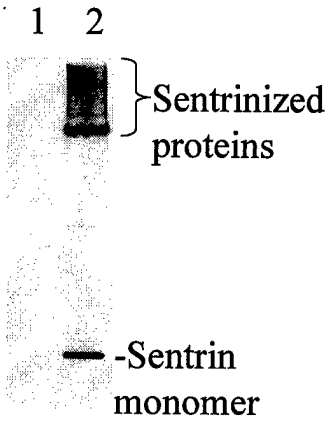

Figure 1. Western blot analysis of HUVEC infected with adeno-β-Gal (lane 1) or adeno-HA-sentrine-1 (lane 2). The position of the sentrin monomer and sentrinized proteins are marked.

ANNUAL PROGRESS REPORT

PROJECT TITLE: "Disaster Relief and Emergency Medical Services Project (DREAMS): Clinical and Basic Science Projects
THE UNIVERSITY OF TEXAS - HOUSTON HEALTH SCIENCE CENTER
PRINCIPAL INVESTIGATOR: Ward CASSCELLS, M.D.
GRANT NO: DAMD17-98-1-8002
REPORTING PERIOD: 11/1/98-10/31/99
Subproject Number/Title:
III B. Mechanisms of cell adhesion and reperfusion injury.

Investigator Name:
Edward T.H. Yeh, M.D.

Investigator Signature:

Progress Report: Describe scientific progress for the quarter in terms of the tasks or objectives listed in the statement of work for this project. Explain deviations where this is not possible. Include data where possible. Present a brief statement of plans or milestones for the next quarter. *Do not exceed two pages.*

Cell adhesion plays a critical role in the attachment of leukocytes in all types of reperfusion injury. Major mediators of this are ICAM-1 (intracellular adhesion molecule-1) and the selectins. In the last period, we have screened for currently approved medical compounds for their ability to inhibit recruitment of inflammatory cells in a mice model that we have validated and published (1). We found that troglitazone, a small molecule that binds to the PPARγ nuclear receptor, can inhibit homing of a monocyte cell line, RAW, to the atherosclerotic plaque. In order to determine the mechanism of action of troglitazone, we used an in vitro culture system, the human umbilical vein endothelial cells, as our model. We showed that a well-known inflammation-inducing cytokine, tumor necrosis factor can induce expression of adhesion molecules, ICAM-1, VCAM-1, and E-selectin on the surface of endothelial cells. Pre-treatment of the cultured human umbilical vein endothelial cells with troglitazone significantly

inhibit tumor necrosis factor-induced ICAM-1 and VCAM-1 expression, but not that of E-selectin. We have further validated these results with other PPARγ agonist and showed that this effect is specific for PPARγ agonists. These novel observations have been published in Circulation (2). We are still dissecting the molecular mechanism whereby PPARg agonist exert its anti-inflammatory effect by examining the NFκB signaling pathway.

1. Patel, S., R. Thiagarajan, J. Willerson, and E. Yeh. 1998. Inhibition of alpha 4 integrin and ICAM-1 markedly attenuate macrophage homing to atherosclerotic plaques in ApoE-deficient mice. *Circulation.* 3:75-81.

2. Pasceri, V., H.D. Wu, J.T. Willerson, and E.T. Yeh. 2000. Modulation of vascular inflammation in vitro and in vivo by peroxisome proliferator-activated receptor-gamma activators. *Circulation.* 101:235-238.

FINAL SYNOPSIS OF *DREAMS I* PROJECTS DIRECTED BY Timothy Scott-Burden, Ph.D.

Introductory remarks: The projects to be summarized comprise II F, III E and III L. Details of results obtained and experimental procedures can be found under the monthly reports (already submitted) for each protocol. Much of the information obtained has facilitated the preparation of the single project proposed for the *DREAMS II* program. The focus of effort into one project (*DREAMS II*) will make better use of the skills in the Vascular Cell Biology Laboratory and thus gives every chance of significant progress being made on the project in terms of completion of animal studies (the appropriate paper work is in preparation), the end point of the protocol. Approval for these animal studies has been obtained from the host institution, The Texas Heart Institute.

II F Foxhole stents to protect cells: This project investigated cell-seeding of metal stents with endothelial cells and smooth muscle cells and subsequent testing of their ability to adhere when stents were subjected to shear stress using a centrifugation assay. Development of the assay system was one aspect of this protocol which has resulted in use by a number of other laboratories for their particular needs. The results from these studies are to be found in the monthly reports, but in summary up to 50% of endothelial cells sloughed-off stents subjected to shear stress levels normally associated with arterial flow. Unlike smooth muscle cells, cultivation of endothelial cells in the presence of sodium ascorbate did not improve adhesion of the latter. Smooth muscle cells adhered well to stents at shear stress levels exceeding those used for endothelial cells (+/- 7% loss) and a significant improvement in attachment (+/- 4%) of myocytes was observed if they were cultured in the presence of ascobate.

The information obtained indicated that smooth muscle cells would provide a good adherend cell lining for stents but the harvest of autollogous cells for this purpose may be problematical in a patient setting. We therefore considered the use of alternative tissue sources for cell harvest and will develop that direction in *DREAMS II.* Another setback on this project was the fact that we were unable to find a commercial source of stents which were "pitted" to provide Foxholes. This is essential for protection of cells during the deployment of the seeded stents. This stymied our efforts to complete the project in respect to implantation of cell-lined stents. At the initiation of this project there were many promises for the provision of stents with pitted surfaces, and therefore this aspect became an unforeseen problem for the completion of the project.

III E Over expression of VEGF in smooth muscle cells: Fallout healing of implanted cardiovascular devices by circulating endothelial progenitor cells has been documented for more than a decade. The purpose of this project was to investigate the possible enhancement of this process by providing a good "seedbed" for the circulating progenitor cells. VEGF shows strong specificity towards endothelial cells, which is not seen with many other growth factors, thus it is an ideal candidate for the task. Over production of VEGF will not enhance growth of other cell types.

We have been successful in cloning bovine VEGF into one of our plasmids of choice and establishing long term (over) expression in smooth muscle cells (see monthly reports). Also, we have looked at expression of VEGF-GFP (green fluorescent protein) fusion protein, again cloned into our vector of choice. The idea behind this work was to

have ways to follow cells that had taken up the tagged growth factor. We have demonstrated that this molecule still retains growth stimulatory properties which are undiminished compared to VEGF alone. Unfortunately we have been unable to carry out the imaging experiments since moneys approved for the purpose of purchasing the required equipment as been coopted for other projects by the *DREAMS I* PI (see reports by Dr. D. Engler). In spite of this we have had some success in seeing apparent uptake of the green VEGF fusion protein by endothelial cells after prolonged cultivation with smooth muscle producer cells.

In other studies we have looked at the effect of monolayers of smooth muscle cells producing VEGF, on the rapidity of endothelial cell attachment and subsequent growth. These assays were compared with control smooth muscle cell monolayers, mock transfected with plasmid devoid of VEGF cDNA. For these studies endothelial cells were tagged with fluorescent probes and monitored hourly by standard photographic methods. This project has yielded a significant amount of information which will be used in the project proposed for *DREAMS II.*

III L. Gene transfer: Manipulating liposome structure: The use of liposomes to effect gene transfer is not as prevalent currently as when this project started. There are a number of newer reagents that mediate the process of transfection with greater efficiency than liposomes. Our early observations regarding direct induction of gene expression by certain liposomes (especially containing cationic functions) has been brought to the attention of the scientific community (meeting presentations and publications). As discussed in the monthly reports, we sort to discover the mechanism(s) of gene induction by cationic liposomes. It is clear that the process differs considerably from that associated with the direct action of cytokines and also slow acting gene induction by endotoxins (see monthly reports). These facts clearly identify this project as a signal transduction project and currently there is not enough available expertise in the Vascular Cell Biology to follow up on this aspect. We have thus clearly identified an interesting and unforeseen role for cationic liposomes but do not think we are able to continue working on the project in the future. What is important is that we have at least alerted people who use cationic liposomes to the possibility of obtaining false or erroneous data. If for example *Lipofectamine*^{TM} is used to transfer NO synthase genes to cells or tissues, a strong positive signal (NO production) may not be due the transgene expression but induction of NO synthase by the direct action of the liposome.

Concluding remarks: Good data from the first two projects have laid the foundation for work proposed in the *DREAMS II* project. Failure to complete project II F is merely a manufacturing problem which was not envisaged when the work was initiated. Promises to provide (obtain) materials did not transpire. Project III E was completed, however additional good data could have been obtained if the budgeted (and approved) imaging equipment had been obtained. As far as project III L was concerned, a measure success was obtained and further work would require a different focus currently not available in the Vascular Cell Biology Laboratory.

Signed:  **Date:** 12/6/99

Annual Report (1998-1999) for DREAMs Projects III-F, -G, -H, -J

PI: D. A. Engler, Ph.D.

Project III-F; Nontoxic Peptide Enhancement of DNA Uptake: Year two progress has been primarily centered around advancing technical objective three of this project, namely: - iii) *to investigate the mechanism(s) of FGF-enhanced gene-delivery to cells.* The previous year's progress helped advance the first two technical objectives to the point where more knowledge about the third technical objective is necessary before much more biologically relevant information can be gleaned concerning this potentially useful form of gene-therapy. Progress on technical objective two, namely - ii) *establishing optimal and reproducible conditions for efficient gene-delivery*, at least from the perspective of the non-cell-based chemical and biophysical characterizations of complex formation conditions that lead to favorable gene uptake, has led us to believe that many of the forms of the molecular conjugates that are present in the molecular population that facilitates DNA uptake by cells would not be useable *in vivo* because of non-specific aggregation phenomenon, and precipitation of gene vehicles. Therefore, delineating the exact mechanisms by which conjugate uptake by cells in this FGF-facilitated DNA uptake phenomenon takes place, using defined experimental conditions - on cell lines that have been genetically modified to help us decipher the role the FGF receptor system plays in this phenomenon, is of paramount importance before further optimization of conjugates can meaningfully take place. To this end most of this year's progress has centered around the construction of such genetically modified cell lines that overexpress the high affinity receptor protein-tyrosine kinase (RPTK) for FGF (see also quarterly reports for project III-J), namely FGFR-1, and the characterization of the FGFR-FGF interaction at the molecular level using the Biacore biosensor in an effort to identify those conditions that influence this interaction (see quarterly reports for periods 5-8), so that we may exploit the power of the FGFR1-CBD fusion protein to help us separate more active complexes (i.e. those forms of FGF-PL-DNA conjugates that bind the FGFR with higher affinity) in an affinity-based chromatographic separation process.

This project will be continued under DREAMS-II funding as both a continuation of technical objective 3, as noted above, and in a manner that will attempt to use an FGFR retargeting strategy to direct insect virus particles (that should display no enhanced tropism for animal cells) toward those genetically modified animal cells that now display increased levels of FGFR-1. We will also attempt to visualize the FGF-mediated DNA conjugate uptake and it's intracellular translocation pathway(s) to the cell's nucleus by microfluorescence imagery, if our request for a fluorescence based digital imaging system that was originally requested in the DREAMS I funding mechanism under project III-H is approved.

Project III-G; Role of FGF-2 in Mutagenesis and Carcinogenesis: Year two progress on this project continued to advance all three technical objectives of this project and continued along the agenda as outlined in last year's ('97-'98) annual report. This project was however restricted in it's scientific progress due to it's heavy reliance on high resolution fluorescence-microscopy imaging, of which this laboratory is currently not physically capable of doing independently, due to the lack of a suitable microscope instrumentation package (planned and budgeted for under project III-H) that was never acquired during this funding period and which was relied upon for advancing these technical objectives further. Even with the instrumental limitations noted, we were able to show that FGF-2 when added in physiological doses (i.e. the amount of factor that could possibly be present at sites of wounding) with either purified exogenous fragments of DNA, or with exogenous naturally liberated DNA fragments from wounded cell extracts (that more closely resembles the form DNA fragments would exist as at sites of injury) to naive cells (representing non-injured

neighboring cell populations at sites of wounding) in culture would increase the nuclear uptake of exogenous fluorescently-labeled marker DNA in a time and dose dependent manner (see period 5 and 6 quarterly reports). These studies also led to a semi-quantitative estimation of the number of nuclei in a cell population affected by this type of treatment; the numbers from which could be used in future estimations of the frequency of pro-mutagenic events in cultured cell populations. Additional studies performed during this time period have also allowed us to establish that the exogenously added DNA fragments do indeed associate with the naive cell's nuclear chromatin in a fairly tight molecular interaction (see period 7 quarterly report), a pre-requisite for any additional recombinatorial cellular process that might result in chromosomal aberrations (mutations) to the naive cell. Furthermore, the type of fluorescence microscopy analysis performed during this time period allowed us to establish that the exogenous DNA that is translocated from outside the cell into the cell nucleus co-localizes in the nucleus with the receptor for FGF, a strong indication that nuclear translocation is possibly mediated by the ternary complex of FGF-2/FGFR/DNA (see period 8 report). The funding levels for DREAMS-II unfortunately do not allow for the continuation of studies related directly to this endeavor. Therefore any future studies to extend these presumably important observations and the impact they will have on wound carcinogenesis will have to await alternate sources of funding. There is currently one manuscript in preparation resulting from these studies.

Project III-H; Luminescent and Fluorescent Molecular Imaging: Effort on this project during this reporting period (year two) was entirely directed at identifying the appropriate type of fluorescence-based microscopy and digital imaging workstation necessary to pursue the identified technical objectives further than what was reported in year one's annual report. Although substantial personnel time and effort on preliminary sub-cellular (and indeed - molecular) imaging experimentation was expended on evaluation instrument set-ups during this reporting period, we have not reported any further substantial progress towards the defined technical objectives of this project other than that reported during period 7 - relating to the spatial resolution capabilities of these fluorescence-based studies on living tissues. As we head into the DREAMS-II funded research, we are still unclear as to the final disposition of our request to obtain this imaging microscope technology, which was approved at the Army level for funding, but is being delayed at the institutional level for intra-institutional re-budgeting purposes to benefit other DREAMS-related projects not directly related to this study. Although studies directly related to the technical objectives of this particular project will not be extended in DREAMS-II, the need for the fluorescence-based imaging station will persist in other DREAMS-related projects and therefore future research may be jeopardized by its absence.

Project III-J; Mechanisms of FGF Signal Transduction: Progress on this project during this year can be summarized as a continuation of our efforts toward technical objective #3, namely iii) *the purification of pp90 for positive identification and subsequent cloning*. Although this technical objective was always expected to be the most labor intensive and problematic, we have been somewhat disappointed in its pace of progress this year. However, as reported in the period 7 report, this protein may be the recently identified FRS-2 molecule based on its partial immunoreactivity with antibodies raised against the FRS-2 molecule. Since our data was inconclusive in this regard we have continued in our original efforts to purify enough of the molecule from natural sources to allow us an unambiguous identification of this molecules identity. As intimated in the report for period 8, we have very recent data from mass spectrometry data on trypsin fragments generated from our gel purified pp90 molecule that suggests that it may be another previously cloned molecule known alternately as alpha glucosidase or the major substrate for protein kinase C known as the 80K-H protein. Although there is still the possibility that our pp90 protein preparation is still contaminated by the protein identified above, and that the pp90 molecule represents such a small proportion of the protein preparation that this tentative identification is erroneous. We are

still in the midst of trying to make this determination based on the levels of matching profiles of our mass spectrometry data to that predicted by database searching. Although this project is not slated for continuation under DREAMS-II, we will continue to pursue these studies until a positive identification is made and leads us in the direction that we need to go to fulfill technical objective #4, that of identifying the role pp90 plays in FGF signal transduction.

ARMY REPORT

Over the past year we have advanced our goals towards defining the presence, regulation and characterizations of the cytochrome P450 subfamily 4F which hydroxylates and deactivates leukotriene B_4 and prostaglandins A_1 and E_1 thereby interrupting the effects of these tissue mediators in promoting inflammation.

The cytochrome P450 4F subfamily has been shown to be present in lung tissue. We have used olignucleotide primers designed to differentiate among the four known cytochrome P450 4F isoforms in reverse transcriptase polymerase chain reaction studies to determine this issue. More recently these results have been extended using Northern Blot analysis.

The control of expression of cytochromes P450 4F is an interesting per contra to the cytochromes P450 4A in that the expression of the 4F forms are suppressed while the 4As are induced by treatment with the Peroxisomal Proliferator Activated Preceptor alpha ({PPAR$_\alpha$) agonist clofibrate. In order to define further the promoter region and enhancer elements, a genomic clone of P450 4F5 has been prepared. Most recently we have been able to establish the identity of the transcription start site using PCR RACe techniques. We have prepared a series of truncations of the 2400 5' flanking region and cloned these truncated sequences in front of a luciferase reporter gene. These reporter constructs will be used to define which of the 5' flanking region cis-acting transcription factor sites figure prominently in the regulation of P450 4F5 expression.

In order to pursue the regulation of these 4F isoforms further, we have cloned with our collaborator Dr. Kawashima, the 4F forms from mouse. We are now characterizing the expression of these forms in wild type mice and in a PPAR knockout mouse strain. We contemplate preparing a 4F knockout mouse strain though we must first decide which 4F isoform should be the target.

Thus far, our work on characterization has utilized animal models. Quite recently we have cloned a new hithertofore unrecognized 4F isoform from a human cDNA expression library. While we still must characterize the sequence for the N terminal 25 amino acid residues, we have enough sequence within the coding region to determine that this clone is different from the two human forms already characterized or those predicted from analyses of the human genome data base.

Thus, our work during this year has accomplished our goals for the year and, moreover, has opened for us the way to additional modalities for defining control and regulation of the 4Fs as well as to assess the utility of the 4Fs in human lung.

H.W. Strobel, Ph.D.

11/24/99

Summary 9/98-10/99: DREAMS Project 2-II **4/15/00**
PI : Richard W. Smalling, MD, Ph.D.

Work on DREAMS Project 2-II, "Mechanisms of Infarct Salvage with Selective Adenosine Infusion In Regional Myocardial Ischemia", began September 1998. Under the direction of Richard Smalling MD, Ph.D. , a team consisting of Michael Rihner MD cardiology fellow, Hela Achour MD volunteer, Ali Denktas MD cardiology research fellow and technicians James H. Amirian, Patty Felli and Carnell Parks we have finished the initial project of two experimental groups and have completed a third arm of Control animals without IC heparin.

Our original experimental Control Group and Adenosine Groups when compared to previous infarction models of similar condtions both revealed increased infarct salvage. Generally we expect an infarct of 55-60 percent in the left ventricle after a two hour occlusion and four hour reperfusion in a control set of experiments . However our Control Group had significant salvage (41%) and the Adenosine Group had reduction in infarct percent zone of risk in the LV (48%).
In our preliminary Dreams Project 2-II heparin boluses were given to keep the infusion wire patent which maybe a confounding factor in determining the efficacy of adenosine in limiting infarct size and reperfusion injury. Heparin had been proven to show protective effects on myocardial tissue independent of its anticoagulent activity. In order to determine the true effect of heparin used in an intermittent small bolus fashion a new control arm with no heparin use was added (and a new adenosine arm with saline flush was proposed). We were able to complete the third arm (Control w/o IC heparin). Further funding was denied for this project in DREAMS 2000 which would have included a fourth group of animals with Adenosine Infusion without IC heparin to keep the infusion line patent.

Currently to date a total of 31 experiments were performed. Work began on the third group (Control with saline flushes of the wire 2/99) and initial experimentation has been completed. All data has been collected and statistical analyis has been performed on the initial two groups . The new Control group work had been completed and also has been compared to the initial two groups. A total of seven final experimental canines per group (21 final data total) has been completed. As expected the new Control group revealed large infarcts (58 %) and this was found to be signifantly different ($p<0.5$) when compared to the original Control group and very close to significant when camparing to the Adensine (heparin flush) Group using ANCOVA . The regional myocardial blood flow data had been completed for the first three groups which showed no significant differences in blood flow throughout the procedure comparing the 3 groups. P-selectin and and ICAM staining expression results is completed for the first two groups and has been submitted for the third group. The third group of animals ICAM-1 and P-Selectin analysis is currently being done and paid for with private funds and results are pending. Tissue samples for

electron microscopy blocked and photgraphed for analysis on the three groups and currently the EM photos are being reviewed by Dr Maximillian Buja, Pathology, DEAN, at the UTH-HSC. using private funds.

Unfortuanately the discontinuation of funding for the DREAMS project in !999-2000 prevented us from finishing the fourth arm of the study (Adenosine w/o heparin flush) at this time. A paper is currently being written on the initial study plus the new Control group by Dr. Ali Denktas MD UTH-HSC Cardiology fellow and should be available for review shortly.

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The Surface of Human AV Grafts Exhibit Thermal Heterogeneity *in vivo*, and Warmer Regions Have Higher Resistance.

Authors: Khawar Gul, Bujin Guo, Morteza Naghavi, Brian Berridge, Ward Casscells, Alan Cohen, James T. Willerson

Institution: University of Texas, School of Medicine Houston TX

We have reported that human carotid plaques, *ex vivo*, have foci of heat, which is largely attributable to macrophages on or near the lumen. Here we asked whether thermal heterogeneity(TH) exists *in situ*.

Methods: 1) In patients(pts) on hemodialysis(HD) for chronic renal failure infrared(IR) photos were taken of the skin overlying the graft and correlated with graft blood flow (QB max) and venous resistance at QB_{250}(VR_{250}). 2) Hemodynamic parameters such as QB max, QB_{250}, and VR_{250} were measured on HD.

Results: All grafts exhibited thermal heterogeneity (33.05°C-35.96°C, mean=34.77 ±. 82. Of 19 pts, 15 had higher temperatures at the venous end (33.69°C-35.96°C, mean=35.03°C ±. 62) as compared to the arterial end (33.05°C-35.96°C, mean=34.64 ±. 88, p=<. 007). We found an inverse correlation between QB max and temperature at the venous end(r=. 50, p=<. 02) Graft temperature related to graft resistance(r=.53, p= <.02)

Conclusion: Thermal heterogeneity in arterial prostheses can be detected by IR camera *in situ* from the luminal side, and from the adventitial side despite flowing blood.

2) Higher temperatures are associated with the venous end particularly those with higher resistance, suggesting that thermal imaging may localize inflammatory stenoses.

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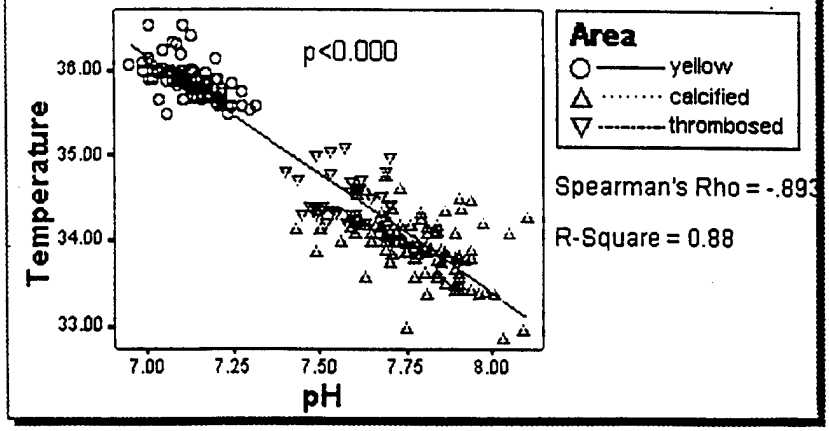
Correlation of Temperature and pH in Living Human Atherosclerotic Plaques

Reji John, Sameh Naguib, Said Siadaty, James T. Willerson, Ward Casscells, Morteza Naghavi

University of Texas Houston, School of Medicine

We have discovered that atherosclerotic plaque are heterogeneous with respect to temperature, and that inflamed plaques with extensive infiltration of macrophages give off more heat than others. More recently we have identified pH heterogeneity in human endarterectomized atherosclerotic plaques (CEA). In this study we have looked for relation between pH and temperature in CEA. Immediately after removal, CEA were kept in a biological media (DMEM) at 37^C for 30 minutes, then removed from DMEM inside a 37^C newborn incubator with humidity above 95%. Using a glass type pH microelectrode and a needle type hypodermic thermocouple electrode mounted on a micromanipulator, we have carefully measured pH and temperature of different points on 8 CEA from 8 patients. At each point, temperature was measured superficially and pH was measured in 200 microns depth. Areas with lower pH have consistently shown higher temperature $r=0.94$, $p<0.001$. Yellow areas containing large lipid core exhibit markedly lower pH with higher temperature whereas calcified regions were associated with lower temperature and higher pH, $p<0.001$. Whether such a correlation exists in vivo, and how much it could be used to locate vulnerable plaque, is yet to be answered.



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In vivo Physiologic Heating of Rabbit Atherosclerotic Plaques Induces Macrophage Apoptosis: A New Approach to Plaque Stabilization.

Authors: Khawar Gul, Morteza Naghavi, Said Saidaty, Ward Casscells, Alan Cohen, James T. Willerson

Institution: University of Texas, School of Medicine Houston TX

Abstract

We have previously shown the effects of gentle heating on the induction of apoptosis in plaque macrophages *ex vivo*. Here we asked whether heating could cause macrophage apoptosis in vivo.

Methods: Watanabe hypercholesterolemic rabbits were fed a cholesterol-rich diet for six months to develop lesions in their aortas. The aorta was then exposed and heated internally (IA), with a cooled ablation catheter (Cardiac Pathways) using a radiofrequency generator (Radionics) at 42° C for 15 minutes. A portion of the aorta was heated from outside (EA). The heated specimens were harvested, then fixed and TUNEL- stained for apoptosis.

Results: In 11 rabbits heated at 42° C for 15 minutes, the percentage of apoptosis for the IA in the intimal layer was 30.3 ± 23.1 and for EA it was 18.6 ± 12.4 as compared to 5.7 ± 8.9 for the control. The Friedman test for simultaneous testing of the three intimal groups was significant at .020 level. For the medial layer, the percentage of apoptosis in the IA group was 2.9 ± 1.56 and for the EA it was 2.6 ± 1.9 as compared to 1.5 ± 2.5 for the control. The Friedman test for simultaneous testing of the three medial groups was not significant at .164.

Conclusion: We conclude that gentle heating of rabbit aorta *in vivo* induces significant macrophage apoptosis in the intimal layer.

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Infrared Fiber Optics for Atherosclerotic Plaques Thermal Imaging

Bujin Guo[1], James T. Willerson[1,2], Greg Bearman[3], Janice McNatt[2], Basit Malik[1], Khawar Gul[2] and Ward Casscells[1,2], 1)Texas Heart Institute, Texas Medical Center, MC 2-255, Houston, TX 77030, 2)The University of Texas, Medical School, Houston, TX 77030 3)ANE Image, 974 East Elizabeth, Pasadena, CA 91104

ABSTRACT

Rupture of atherosclerotic plaques–a major cause of heart attack and strokes–is not easily predictable. Treadmill stress tests fail to detect many persons at risk. Inflammation is characterized by heat, swelling, redness and pain. Fatal plaques are associated with activated inflammatory cells. We have demonstrated *in vitro* that heat accurately locates the plaques that are significantly warmer than atherosclerotic plaques without inflammation. To develop a non-surgical method of locating these plaques, a novel infrared fiber optic imaging system has been developed. The infrared fiber optical imaging bundle consists of an array of 100 μm individual As_2S_3 chalcogenide glass fibers which transmit infrared radiation from 0.7 μm to 7 μm with little energy loss. The first prototype consists of a 30×30 (6 mm O.D.) square array and the second consists of a 10×10 (3 mm O.D.) square array. By combining the infrared fiber bundles with a highly sensitive Indium Antimonide (InSb) infrared focal plane array (FPA) detector, we are able to obtain *in vivo* thermal images in the aortas of Watanabe hereditable hypercholesterolemic rabbits. These images have clearly shown temperature differences over a 2 mm^2 area, with ~100 μm spatial and better than 0.1 °C thermal resolution. We conclude that 1) thermal heterogeneity of atherosclerotic plaques is detectable *in vivo* and 2) an infrared catheter may be useful for studying the causes and natural history of heterogeneity of plaque temperature and eventually for localization and therapeutic treatment of vulnerable plaques.

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Near Infrared Spectroscopic Characterization Living Human Carotid Atherosclerotic Plaques

Jing Wang,[1] Bujin Guo,[1] Tomas klima,[1] James D. Willerson,[1,2] Ward Casscells[1,2]

1 Texas Heart Institute; 2 Division of Cardiology, Department of Internal Medicine, University of Texas-Houston Medical School,

Arteriosclerotic plaques consist of thrombus, inflammatory and smooth muscle cells, lipid-rich materials, fibrous tissues and calcification. Currently there is no practical technique to determine the composition-related vulnerability of the plaques in the clinical setting. Ultrasound imaging is presently the prevailing method for clinical diagnosis of arteriosclerosis in the carotid arteries, but it cannot yet be used to predict the vulnerability to rupture of plaques with 50-100 μm thin cap. Near-infrared (NIR) spectroscopy, permits inference about the biochemical composition of tissue. The NIR spectrum of biological specimens in the spectral region 700 to 2500 nm is complex, but improved instrumentation and sophisticated multivariate chemometric data analysis techniques, now permits interpretations from complex NIR spectra. We hypothesised that factors, such as fat, hypoxia, heat, glucopenia, tissue pH, etc., would together change the NIR spectral signature sufficiently to enable the identification of vulnerable plaques. With the approval of the Institutional Review Board of the Texas Heart Institute and St Luke's Episcopal Hospital, Houston, Texas, we have studied to date 14 human carotid atherosclerotic plaques from 14 patients using NIR spectroscopy. A total of 120 NIR spectra were recorded from these plaques and analyzed using spectral classification technique. We were able to identify significant NIR spectral differences between the various arteriosclerotic plaques, based on the composition identified from, and correlated to, the tissue histology obtained by conventional methods. We believe that the compositional information extracted from this investigation may be useful in the development of a new diagnostic methodology to predict the vulnerability of certain plaques to rupture.

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Ultrasonically Heated Stent: A new approach to thermal therapy of atherosclerotic plaques and, prevention and treatment of restenosis

Morteza Naghavi, Birendra Lal, Bujin Guo, Khawar Gul, James T. Willerson, Ward Casscells

University of Texas Houston, School of Medicine

Anti-proliferative and apoptotic effects of hyperthermia have long been known, particularly in cancer treatment. We have shown that gentle heating reduces inflammation in atherosclerotic plaques by suppressing pro-inflammatory cytokines and promoting apoptosis of macrophages. Others have recently identified the inhibitory effect of low level heating on SMC proliferation after balloon injury. Ultrasound (US) hyperthermia is widely used in cancer therapy. US waves heat subjects based on their absorption and reflection rate. The latter may explain the excessive heating effect of US on the surface of bone. We therefore hypothesized ultrasonically heated stent can be made with certain material that produces more heat than surrounding tissue when irradiated by US. We have used a number of non-toxic materials in our phantom to test the hypothesis. The phantom is a 10CM thick layer of pork muscle, inside which different annular stent shape materials are placed for a given experiment. A 2.5 CM US probe was fixed perpendicularly above the surface of the phantom to mitigate non-invasive application of US. Temperature was continuously monitored by multiple hypodermic thermocouples and infrared camera. We applied different FDA-approved levels of therapeutic ultrasound, (intensity 0.5-2.5 W/CM2, frequency 1-3 MHZ), in both pulse and continuous modes. We found that the temperature of stent-shaped materials made of polymers such as polyurethane, silicon, and some types of PVC increases (2-35 C) more and (1.5-15 times) faster than temperature of surrounding tissue. However, other polymers such as Teflon, Lexan, PTFE, and some other kinds of PVC failed to show a selective thermal effect. We have also observed a small heating effect of US waves most likely due to reflection on tissue immediately surrounding a metal stent (2C increase after 15 min). **Conclusion:** US heating of tissue adjacent to a prosthesis varies with composition. The technique was proven useful in preventing stenosis of stents and vascular grafts. However, the material and design of choice for the stent, the *in vivo* thermal / non-thermal effect of focused / unfocused US on the stented artery and its surrounding tissues, and finally its efficacy and adverse effects in comparison with other competing technologies for non-invasive heat delivery (radiofrequency, MR,...) remain to be answered.

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Angiographic Predictors of Plaque Progression in Mildly to Moderately Diseased Coronary Arteries

Moein F. Vaseghi, Khaled Hassan, Said Siadaty, Morteza Naghavi, Richard L. Kirkeeide, Ward Casscells

University of Texas Houston, School of Medicine

Progression of atherosclerotic plaques has not been predicted by angiography. We hypothesized that progression of plaques creating <50% stenosis is predicted by fresh mural thrombus, lesion location at a branch point, and plaque blush – delayed clearance of contrast possibly due to angiogenesis or cap fissuring. **Methods:** Films of 200 patients undergoing repeat angiography for symptoms of ischemia, 5.6±4.8 (mean±SD) months apart, were reviewed by two blinded observers. 123 patients were excluded due to prior PTCA or CABG, initial lesion severity >50%, and non-comparable paired angiograms. Presence of plaque blush, calcification, clot (mobile defect), eccentricity, and branch point location were compared in progressing (• 20% stenosis increase) and non-progressing plaques. **Results:** 16 lesions in 15 patients progressed from 29±13% to 68±14% over 8.1±7.9 months. Patients with and without progression were similar in gender mix, age, CHD risks, medications, days between angiograms, clinical presentation and initial stenosis severity. Logistic regression identified plaque blush (p=.002), calcification (p=.024) and a branch point location (p=.001) as the predictors of plaque progression. Using these signs, the model predicted the odds ratio for plaque progression (ORp) as: $ORp = e^{2.5*BL + 1.8*CA + 2.6*BR}$. The model has a 81% sensitivity, 77% specificity and an overall accuracy of 78% when an ORp of 1/3 was used to classify the groups. In other words, a moderate (<50%) stenosis with both "blush" and branch point signs had a 25% chance of progressing within 8 months if calcified as well. Such lesions had a 100% likelihood of progressing but only 40% of progressing lesions had all 3 signs. **Conclusion:** In mild to moderate coronary stenoses, plaque blush (a novel sign) branch point location and calcification are predictive of plaque progression. If confirmed by prospective analyses, these criteria may be helpful in clinical decision making.

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Heat Down-Regulates Tumor Necrosis Factor-α and IL-6 in Human Atherosclerotic Plaques

Birendra N. Lal, Tarun Tewatia, Mark L. Entman, Keith A. Youker, Said Siadaty, James T. Willerson, Ward Casscells, *University of Texas at Houston, Texas*

We recently observed that in living human carotid endarterectomy(CEA) specimens, plaques that are warmer than others(by1-3°C) undergo cooling after exposure to heating (40°C-42°C X 15 min), suggesting that heat has an anti-inflammatory action. Therefore, we asked whether heat might regulate tumor necrosis factor-α and IL-6, pro-inflammatory cytokines that promote macrophage proliferation and survival and endothelial cell dysfunction, thrombosis and apoptosis. Seven living, freshly harvested human CEA specimens were divided into two parts, one piece of which was placed at 37°C for 6.3 hrs and other at 42°C for 15 min, followed by six hrs at 37°C, in Dulbecco's modified eagles' medium in a 5% CO_2 incubator. The specimens were fixed, processed and immunostained, using antibodies to TNF-α,IL-6, α-actin and HAM-56. Visual inspection and quantitative morphometry showed a marked decrease in TNF-α immunoreactivity of plaque α-actin positive cells and macrophages (mean 4.42± 2.33SD at 37°C Vs 1.5 + 2.1 at 42°C, P=0.04). Most of the TNF-α immunoreactivity was found in the α-actin positive cells and the reduction of TNF-α by heating was seen primarily in those cells. Thus heating at 40°C-42°C decreases TNF-α and IL-6 immunoreactivity in freshly harvested human carotid plaque. We hypothesized that heat may function to down-regulate macrophage pro-inflammatory activity. The possible therapeutic potential of gentle, non-lethal heating of plaque is being investigated.

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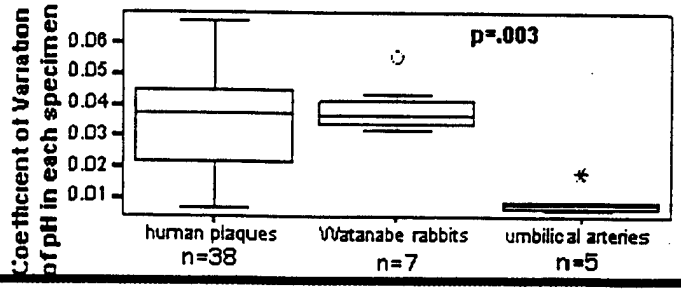
pH Heterogeneity of Human and Rabbit Atherosclerotic Plaques

Reji John, Sameh Naguib, Said Siadaty, KC Kurian, Roxana Grassu, Mark Snugg, Barry vanWinkle, James T. Willerson, Ward Casscells, Morteza Naghavi

University of Texas Houston, School of Medicine

As atherosclerotic plaques are heterogeneous with respect to inflammation, calcification, vascularity, oxygen, and temperature, we hypothesized that they vary in pH, and that the variation might be useful in predicting progression / instability of the plaque. Using a glass type microelectrode mounted on a micromanipulator in a 37^{C} incubator, we measured pH of human carotid endarterectomized atherosclerotic plaques (CEA) immediately after removal from 43 patients. We used dual emission fluorescence ratio imaging microscopy (FRIM) using a pH-sensitive probe (BCECF) in plaques to validate the method. Temperature and oxygen content of CEA were also monitored. The same procedures were used to study 5 human umbilical arteries (HUA) and 7 Watanabe hypercholesterolemic rabbits (WHR). pH measured in 661points of 38 CEA was 7.70±.46, in 176 points of 7 WHR was 7.44±.45, and in 113 points of 5 HUA was 7.19±.08. In CEA, pH of yellow areas containing large lipid cores, were significantly lower than pH in calcified and thrombosed areas (7.44±.46 vs 7.9±.36, p<.000). Heterogeneity of pH in CEA, WHR, and HUA were .035±.014, .039±.008, and .009±.0005 respectively (p=.003). FRIM confirmed microscopic pH heterogeneity in both humans and rabbits. Therefore, living atherosclerotic plaques exhibit marked pH heterogeneity, with lower pH in areas with lipid core and higher pH in calcified regions. The source of pH heterogeneity, its impact on stability of plaques particularly through acidic matrix-degrading enzymes, and its potential clinical utility in locating vulnerable plaques, remain to be determined. A spectroscopic catheter is being developed to characterize pH, oxygen, and other potential markers of vulnerability, in vivo..



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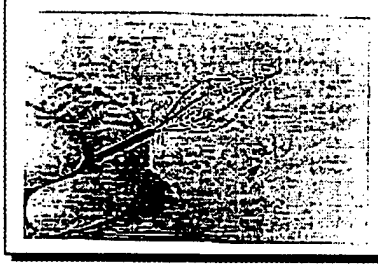
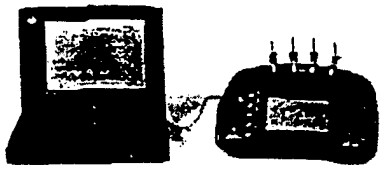
Thermosensor Catheter; a nitinol shape memory basket catheter to measure temperature of vessel wall with continuous blood flow

Morteza Naghavi, Said Siadaty, James T. Willerson, Ward Casscells

University of Texas Houston, School of Medicine

We have previously correlated temperature of atherosclerotic plaque with histopathological characteristics of vulnerability to rupture. We found that inflamed unstable plaques give off more heat. Others have recently correlated plaque temperature with patients' clinical presentation. In order to monitor temperature of vessel wall without occluding blood flow we have developed an intravascular thermosensor catheter. Here we report the specifications of the catheter along with proof of principle in our canine model of atherosclerosis. The system comprises a 3F catheter, an ultra-thermometer, and a PC for thermographic imaging. An expandable basket is made of 4 hollow nitinol wires (ID:0.006", OD:0.008") with 0.003" thermocouple wires built-in at the end of a hollow shaft connecting the basket to the ultra-thermometer and carrying insulated thermocouple wires. The system has a temperature resolution of 0.005C and spatial resolution of 0.5 mm with 0.01 sec acquisition time. The thermocouple sensor inside the nitinol wire is insulated from internal wall of the wire and therefore is less affected by blood temperature. Upon release from a guiding or a delivery catheter, the basket opens, expands and contacts the wall to measure its temperature. The number and size of wires in the basket can be modified to pass through a guide wire lumen of an angioplasty catheter allowing measurement of temperature before and after occlusion of blood flow. We have, thus far, tested the catheter in 5 atherosclerotic canine peripheral and coronary arteries. The catheter detected significant temperature heterogeneity in areas with lesions and no complications have been noted.





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IN VIVO PHYSIOLOGIC HEATING OF RABBIT ATHEROSCLEROTIC PLAQUES INDUCES MACROPHAGE APOPTOSIS: A NEW APPROACH TO PLAQUE STABILIZATION.

THE UNIVERSITY OF TEXAS HOUSTON HEALTH SCIENCE CENTER

Khawar Gul, Morteza Naghavi, Said Siadaty, Ward Casscells, Alan Cohen, James T. Willerson

University of Texas, Medical School, Houston TX

ABSTRACT

We have previously shown the effects of gentle heating on the induction of apoptosis in plaque macrophages *ex vivo*. Here we asked whether heating could cause macrophage apoptosis *in vivo*.

Methods: Watanabe hypercholesterolemic rabbits were fed a cholesterol-rich diet for six months to develop lesions in their aortas. The aorta was then exposed and heated internally, with a cooled ablation catheter (Cardiac Pathways) using a radiofrequency generator (Radionics) at 42° C for 15 minutes. The heated specimens were harvested, then fixed and TUNEL-stained for apoptosis.

Results: In 11 rabbits heated at 42° C for 15 minutes, the percentage of apoptosis in the intimal layer in the internally heated aorta was 30.3 ± 23.1 as compared to 5.7 ± 8.9 for the control. Friedman test was significant at .020 level. For the medial layer, the percentage of apoptosis was 2.9 ± 1.56 in the internally heated aorta as compared to 1.5 ± 2.5 for the control. Friedman test was not significant at .164.

Conclusion: We conclude that gentle heating of rabbit aorta *in vivo* induces significant macrophage apoptosis in the intimal layer.

INTRODUCTION

Atherosclerosis is an inflammatory disease. Atherogenesis has been considered by many to consist largely of the accumulation of lipids within the artery wall; however, it is much more than that. In fact, the lesions of atherosclerosis represent a series of highly specific cellular and molecular responses that can best be described, in aggregate, as an inflammatory disease.

The lesions of atherosclerosis may be present throughout a person's lifetime. In fact, the earliest type of lesion, the so called fatty streak, which is common in infants and young children, is a pure inflammatory lesion, consisting only of monocyte-derived macrophages and T lymphocytes. By asking questions about arterial inflammation, we may be able to gain insight into the process of atherogenesis. The ubiquitous monocyte, the precursor of macrophages in all tissues, is present in every phase of atherogenesis.

Elevated body temperature is known to enhance both inflammation response and immune function. One of the important effects is expression of heat shock proteins (HSP). Heat shock proteins, especially hsp 70, have protective effects in inflammation. Inflammation results from the recruitment and activation of macrophages. These proteins in turn have the ability to protect cells and tissues from the deleterious effects of inflammation. HSP (hsp 70) may also exert protective effects in the immune system by contributing to the processing and presentation of bacterial and tumor antigens. Heat, hence, has a therapeutic application. Heat has also known to be of beneficial effect in the treatment of cancers specially the hepatic carcinomas.

Apoptosis, a term introduced by Kerr et al, differentiates a special type of cell death from necrosis resulting in cell shrinkage, condensation of nuclear chromatin and ultimately engulfment of apoptotic body by surrounding cells. Cell death plays a major role in the organization of the cell associations that we call tissues. This usually elicits an inflammatory reaction, itself capable of inflicting more cell damage and at the same time initiating digestion of the dead cells by neutrophil polymorphs. Sometimes death is conspicuous but essential for normal regulation of tissue cell number. Programmed cell death falls into this category. There is no inflammatory reaction. We have shown, previously, that macrophages in the human carotid plaques undergo selective apoptosis when exposed to temperatures of 42°C for 15 minutes *ex vivo*. The smooth muscles have not shown significant apoptosis at this temperature. We, therefore, hypothesized that *in vivo* heating of the plaques at this temperature will reduce the burden of inflammation, subsequently leading to stabilization and remodeling. We also hypothesize that macrophages are more sensitive to apoptosis than smooth muscles cells.

METHODS AND MATERIALS

With the approval of the Institutional Review Board of the Hermann Hospital and University of Texas Medical School, we did our experiments on 11 Hypercholesterolemic Watanabe Rabbits, who were fed a cholesterol rich (1%) for 6 months.

Instruments

A 7F Cooled Tip Ablation Catheter (Electrophysiology Catheter) model # 3005 acquired from Cardiac Pathways Corporation, Sunnyvale, California was used for IA heat delivery. We used a Lesion Generator model # RFG-3E acquired from Radionics, Burlington MS in order to deliver RF signals to the tip of the catheter. The Cooled Tip Ablation Catheter was hooked up to the RF Generator for the RF signal. In order to cool down the tip of the catheter, an infusion pump was used and normal saline 15°C was run through the tip continuously.

Phantom

One of our major concerns was to find out the power of RF signal that would generate the desired temperature to deliver to the walls of aorta. To choose the desired delivery of RF signal, we designed a simulated model of an artery.

Our phantom model consisted of the Cooled Tip Catheter attached to the RF Generator and connected to an infusion pump. The tip of the catheter was then passed through the simulated model. Two thermisters were placed close to the tip of the catheter, about 10-mm apart from each other. The RF Lesion Generator was set to deliver current of 2, 3 and 4 watts/cm^2 for 15 minutes. The temperature was recorded from both the thermisters at 60 seconds.

Table 1 Simulated Heating Model

Time (seconds)	Power (watts/cm^2)	Temperature (RF Generator)	Temperature (Thermister A)	Temperature (Thermister B)
60	4	40	39.6	34.2
120	4	41	39.9	34.7
780	4	44	43.5	41.5
840	4	44	43.6	41.8
900	4	44	43.6	42.1

Table showing results of heating a simulated model

We chose power at 4 watts/cm^2 for the in vivo study.

Animals

Cholesterol fed, hereditary Hypercholesterolemic adult Watanabe rabbits, both male and female, weighing 3-3.5 kg and between the ages of 16 and 18 months were used as the model to test the hypothesis. These rabbits were fed high cholesterol diet for at least 6 months to produce Atherosclerotic lesions in their arteries.

Heating Procedure

Rabbits were anesthetized and a left thoracotomy was performed to expose the aorta. The core temperature of the rabbit was continuously monitored. An area on the surface of aorta was identified and marked with indelible ink. Tip of the catheter was placed on that area and RF signals delivered for 15 minutes at 42°C and 4 watts/cm2. After the external heating, a small incision was made in the aorta and the tip of the catheter was inserted in the aorta, then advanced into the arch. RF signal was delivered at 4 watts/cm2 for 15 minutes. The temperature of the vessel wall was confirmed by inserting a thermister inside the lumen and also by infra red thermography. After the heating procedure, rabbits were left anesthetized for another 2 hours, then euthanized at the end of that period. The thoracic cavity was opened and the location of the tip of the catheter identified. That area was marked with indelible ink. The specimens were removed from the aorta and separated into three different parts: Control, externally heated and internally heated. The specimen were stored in 10% Formalin. Slides were prepared by the Pathology Department at Texas Heart Institute and stained for TUNEL assay.

Method of detection of Apoptosis

TUNEL assay was done to quantitatively determine apoptosis in the specimen. We analyzed each specimen in two histological areas: intima and media. In each of this layer, we counted the total number of normal and apoptotic macrophages as well as apoptotic non- macrophage cells using light microscopy. In order to confirm the results of TUNEL assay, we also used electron microscopy on few slides.

Table 2 Apoptotic Cell Density in Sub Groups (n=11)*

Group	Mean	Std Deviation
Control	5.68	±8.89
Internal Heating	30.29	±23.07
External Heating	23.07	±12.41

p value by Friedman Test = .02 suggests Internally and Externally Heated Groups are statistically different than apoptotic cells in Control Group.

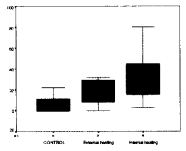
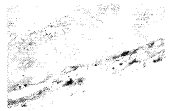


Table III Wilcoxon Signed Ranks Test (for paired testing)

Pairs	P value
Control – Internal Heated Group	.01
Control – External Heated group	.03
Internal – External Heated Group	.155

Slide showing Intima of Aorta in the Control Group



Slide showing Intima of Aorta in Heated group

DISCUSSION

There are limited data regarding effects of hyperthermia on macrophages and even less in those located in blood vessel walls. The effects of in vivo heating have not been shown before. The present study evaluates the effects of gentle heating on macrophages and non-macrophage cells in the intima and media in rabbit aorta.

From our experiments in a rabbit model, we have found that gentle heating induces apoptosis in macrophages in the intimal layer. Macrophages produce a number of factors, such as bFGF and VEGF that can stimulate proliferation of endothelial cells, and it is likely that these have pro-angiogenic activity in situations, such as wound healing. Thus, apoptosis is a protective mechanism that removes macrophages from the foci of atherosclerotic plaque. The disappearance of macrophages by apoptosis could have a positive effect on plaque stabilization and remodeling. Indeed, the death of macrophages should lead to decreased breakdown of collagen fibers. The induction of apoptosis also helps control inflammation by removing macrophages that secrete the mediators of inflammation.

The results of our experiments provide a potential mechanism for destruction of macrophages, thus controlling their accumulation in tissues. The reason for the temperature-associated sensitivity of macrophages to apoptosis is unclear. One possibility is that the protection from programmed cell death may be the result of a temperature sensitive agent. Another possibility is that hyperthermia reduces the expression of the bcl-2 gene or destroys its product. Since bcl-2 in some cells blocks programmed cell death, reduction of its intracellular concentration could favor apoptosis.

Monocyte-derived macrophages secrete cytokines, chemokines, growth-regulating molecules, and metalloproteinases and other hydrolytic enzymes. The replication and survival of mononuclear cells in the lesion depends on factors, such as macrophage colony stimulating factor and granulocyte-macrophage colony stimulating factor for monocytes and interleukin-2 for lymphocytes. The exposure to these factors permit macrophages to survive and multiply in the lesions. On the other hand, cytokines such as interferon-γ activate macrophages and under certain circumstances induce them to undergo programmed cell death (apoptosis). In vivo, macrophages may become involved in the necrotic cores characteristic of advanced, complicated lesion.

Initially, the only cells thought to proliferate during expansion of atherosclerotic lesions were smooth-muscle cells. However, replication of macrophages and T cells is probably of equal importance. The ability of macrophages to produce cytokines (tumor necrosis factor , interleukin-1, and transforming growth factor), proteolytic enzymes and growth factors (platelet growth factor and insulin-like growth factor I) may be critical in the role of these cells in the damage and repair that ensue as the lesions progress. Further, activated macrophages in plaque express class II histocompatibility antigens such as HLA-DR that allow them to present antigens to T lymphocytes. Thus, it is not surprising that cell-mediated immune responses may be involved in atherogenesis, since both CD4 and CD8 T cells are present in the lesions at all stages of the process. Controlling the number and reaction of macrophages might alter these responses favorably.

The cellular interactions in atherogenesis are fundamentally no different from those in chronic inflamatory-fibroproliferative diseases, such as cirrhosis, rheumatoid arthritis, glomerulosclerosis, pulmonary fibrosis, and chronic pancreatitis. In most patients, myocardial infarctions occur as a result of erosion or uneven thinning and rupture of the fibrous cap, often at the shoulders of the lesion where macrophages enter, accumulate, and are activated and where apoptosis may occur. Stable advanced lesions usually have uniformly dense fibrous caps. The potentially dangerous lesions are often non-occlusive and thus difficult to diagnose by angiography, yet at autopsy active inflammation is evident in the accumulation of macrophages at sites of plaque rupture. Macrophage accumulation may be associated with increased plasma concentrations of both fibrinogen and C-reactive protein, two markers of inflammation thought to be early signs of atherosclerosis. Plaque rupture and thrombosis may be responsible for as many as 50 percent of cases of acute coronary syndromes and myocardial infarction. Thus, altering macrophage response may stabilize plaque and could be important in preventing myocardial infarction and strokes caused by plaque rupture.

CONCLUSION

1. Gentle Heating of the aortic wall of hypercholestrolemic Watanabe rabbits at 42°C for 15 minutes *in vivo* causes controlled, selective apoptosis in macrophages.
2. Significant apoptosis was not seen in the smooth muscle cells in the medial layer.
3. Further experiments are needed to understand and utilize this mechanism more efficiently. This may be a potential therapy for unstable atherosclerotic plaques.

The Surface of Human AV Grafts Exhibit Thermal Heterogeneity in vivo, and Warmer Regions Have Higher Resistance.

THE UNIVERSITY OF TEXAS HOUSTON HEALTH SCIENCE CENTER

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ABSTRACT

We have reported that human carotid plaques, *ex vivo*, have foci of heat, which is largely attributable to macrophages on or near the lumen. Here we asked whether thermal heterogeneity(TH) exists *in situ*.

Methods: 1) In patients(pts) on hemodialysis(HD) for chronic renal failure infrared(IR) photos were taken of the skin overlying the graft and correlated with graft blood flow (QB max) and venous resistance at QB_{250}(VR_{250}). 2) Hemodynamic parameters such as QB max, QB_{250}, and VR_{250} were measured on HD.

Results: All grafts exhibited thermal heterogeneity (33.05°C-35.96°C, mean=34.77 ±. 82. Of 19 pts, 15 had higher temperatures at the venous end (33.69°C-35.96°C, mean=35.03°C ±. 62) as compared to the arterial end (33.05°C-35.96°C, mean=34.64 ±. 88, p=<. 007). We found an inverse correlation between QB max and temperature at the venous end(r=. 50, p=<. 02) Graft temperature related to graft resistance(r=.53, p= <.02)

Conclusion: Thermal heterogeneity in arterial prostheses can be detected by IR camera *in situ* from the luminal side, and from the adventitial side despite flowing blood.

2) Higher temperatures are associated with the venous end, particularly those with higher resistance, suggesting that thermal imaging may localize inflammatory stenoses.

INTRODUCTION

Patients with end-stage renal failure undergoing hemodialysis require a long lasting site for vascular access. A-V grafts provide vascular access im nay individuals, but failure of arteriovenous prostheses (grafts) typically occurs over time. Grafts usually last at most a few years before failing. Graft failure necessitates repeat surgery at a different venous site. Since the number of venous sites is limited, anything that prolongs graft life also prolongs the life of the patient. The incidence of venous stenosis in the first postoperative year varies from 50-60% in hemodialysis A-V grafts. This process has been called accelerated atherosclerosis, though some differences may be found between this and the failure of venous bypass grafts to the heart or atherosclerosis in the native coronary and carotid arteries. Thrombosis is the leading cause of vascular access failure, and usually results from stenotic lesions lesions at the venous anastomotic area. These lesions develop from progressive neointimal hyperplasia downstream from the anastomosis leading to profound proliferation of smooth muscle cells, which has also been continuously found in typical atherosclerotic lesions. Inflammation is a contributory factor in venous stenosis. It manifests in different ways such as heat, pain and discomfort at the site of inflammation. Thus any approach that can pick up tell tale signs of inflammation, helps in early detection of imminent graft failure.

We hypothesized that surface temperature over these grafts relates to their functioning and survival. Moreover, thermal heterogeneity over these grafts predicts graft failure. We also determined whether thermal heterogeneity correlates with the hemodynamic measurements

PATIENT CHARACTERISTICS

The patients for this study were enrolled from the Hemodialysis Unit at Memorial-Hermann Hospital and from GAMBRO Clinic, a privately owned hemodialysis center.

Total number of patients:	19
Average Age:	46 years
Gender:	
Male:	15
Female:	4
Average Age of AV Graft:	26.4 months (9-60 months)

METHODS AND MATERIALS

With the approval of Institutional Review Board of the Hermann Hospital and University of Texas, we imaged AV Grafts of 19 patients using an Infra Red Camera. All of these patients had come for scheduled hemodialysis at our center.

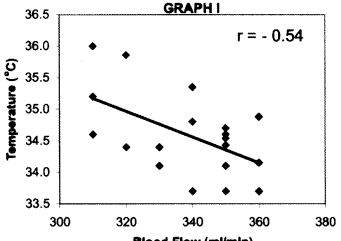
THERMOGRAPHY

The patients coming to hemodialysis unit were contacted and the procedure was explained to them. After they underwent hemodialysis, an infra red image was taken from the arm where the graph was placed. The image was taken in three different positions: arm raised, arm lowered, blood flow occluded by applying the sphygmomanometer cuff. An image was taken from the other arm as a control measurement.

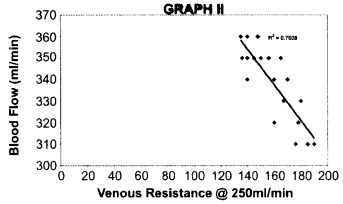
RESULTS

Table I Hemodynamic Measurements Obtained On Hemodialysis

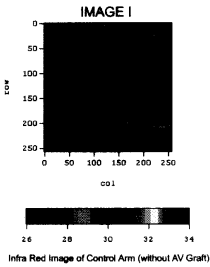
#	Arterial Temperature	Venous Temperature	Δ	Age (yrs)	Age of Graft(mos)	QB(max)	VR@QB250
1	35.0	33.7	-2.10	43	20	340	140
2	34.2	35.9	1.70	45	36	320	160
3	34.4	35.4	1.00	32	16	340	170
4	33.6	35.2	1.60	35	54	310	176
5	33.2	34.2	1.00	49	16	360	148
6	35.1	34.4	.70	36	12	350	156
7	34.2	34.5	.30	35	9	360	140
8	34.3	34.9	.60	54	16	360	140
9	34.3	34.8	.50	34	12	340	160
10	34.7	33.7	-1.00	45	28	360	135
11	33.8	34.1	.30	51	36	330	180
12	34.8	34.7	-.10	35	24	320	165
13	33.5	33.7	.20	60	36	360	145
14	35.8	36.0	.20	64	60	310	190
15	34.3	34.4	.10	43	24	330	167
16	33.4	34.6	1.20	32	9	310	185
17	33.9	34.4	.50	39	24	320	178
18	34.4	34.1	-.30	44	28	360	150
19	34.6	34.6	.00	35	18	350	136
		Ave	.27	40	26.4	338.4	159



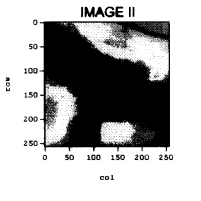
Graph showing correlation between Maximum Blood Flow (Qbmax) and Temperature over the Venous End



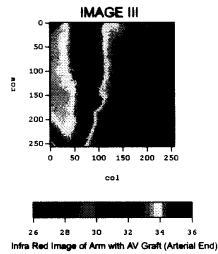
Graph showing correlation between Maximum Blood Flow (Qbmax) and Venous Resistance at $QB_{250ml/min}$



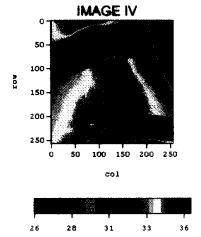
Infra Red Image of Control Arm (without AV Graft)



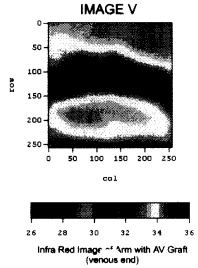
Infra Red Image of Arm with AV Graft (Arterial End)



Infra Red Image of Arm with AV Graft (Arterial End)



Infra Red Image of Arm with AV Graft (venous end)



Infra Red Image of Arm with AV Graft (venous end)

DISCUSSION

The development of stenosis is thought to be a function of vessel injury to vein and artery at the time of surgery as well as injury to vein by the high flows present during hemodialysis. The graft elicits a foreign body an inflammatory and thrombotic response. These effects can be targeted therapeutically in other areas of atherosclerosis. Nevertheless, despite aspirin therapy, thrombosis often progresses. There is danger using anti-inflammatory therapy in these patients who are already immunocompromised. At present there is no proven medical treatment for the prevention of graft failure.

Our study of patients with A-V prostheses detected thermal heterogeneity, venous ends were hotter than the arterial ends. We also obtained hemodynamic measurements such as maximum blood flow (QB max), and Venous Resistance at $QB_{250ml/min}$ on hemodialysis. There was an inverse correlation between the Maximum Blood Flow and Temperature over the venous end. We also saw an inverse correlation between maximum blood flow and Venous resistance.

CONCLUSION

We conclude from this study that we may be able to detect imminent graft failure utilizing thermal heterogeneity as well as the hemodynamic parameters upon hemodialysis. Inflammation manifests as heat and an infrared camera can be used to pick up warmer regions which may over lie the inflamed areas. This may predict the development of stenotic regions in the graft prostheses. The traditional methods such as Doppler ultrasound flow used to predict graft failure have not shown to be accurate. We have used inflammation as a criteria to identify the compromised grafts.

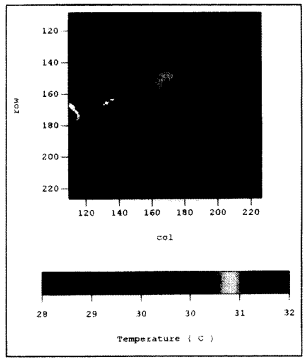
Thermosensor Basket Catheter For Thermal Detection of Atherosclerotic Plaques

Morteza Naghavi, Said Siadaty, Khawar Gul, Sameh Naguib, James T. Willerson, Ward Casscells.

The University of Texas-Houston, Medical School, Division of Cardiology

BACKGROUND

Our group is credited for the discovery of temperature heterogeneity of human living atherosclerotic plaques ex-vivo.[9]
More recently others have supported our findings in vivo using a single channel thermal detecting catheter.[10]
We have been developing an infrared thermal imaging catheter which is currently under construction.
Since infrared technology demands significant technological challenges with some cost issues, and the catheter yet to be commercialized, we have designed a thermosensor basket shape expandable catheter to address the thermal monitoring of atherosclerotic plaques.



Infrared photograph of a rabbit atherosclerotic plaque showing as a hot spot

AIM

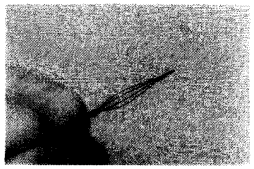
Develop a multi-channel thermosensor catheter to measure temperature of vascular wall with continuous perfusion.

DESIGN

As shown in the figures the basket has three parts, 1) 4 expandable nitinol wires in the form of basket placed at the end of the catheter containing 4 thermosensors, 2) a hollow nitinol tube which carries thermosensor wires and connects a thermometer. The tube has another temperature sensor at the end to simultaneously monitor blood temperature, 3) an ultrathermometer for recording the temperature. We have also placed another thermal sensor at the very end

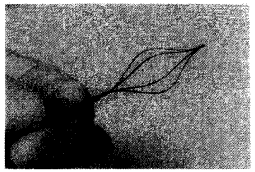
Table of Specifications

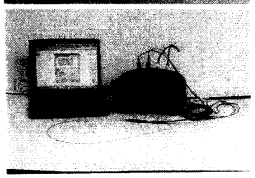
- Thermal resolution (sensitivity):	= <0.001 C
- Spatial resolution:	<0.5 mm
- Accuracy:	<0.1 C
- Sampling rate:	> 4 per each electrode
- Size of basket	multiple sizes (2F for coronary, and 4F, 8F, 10F
	for aorta and peripheral arteries
-Width to length ratio	> 1.7
-Nitinol wire and shaft	hollow with accepted flexibility
-Number of sensors	> = 4



PROTOTYPE I

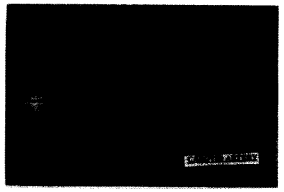
In prototype (I) we have used 4 thermocouple sensors placed on 4 lead expandable nitinol wires. A nitinol hollow tube connects the basket to an ultrathermometer to record the temperature as shwon in the figures ... (photographs of prototype one)

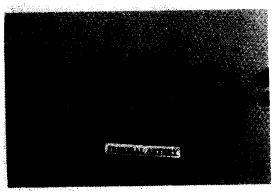


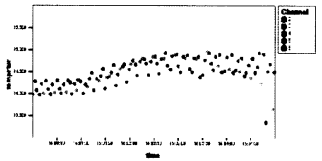


FINDINGS

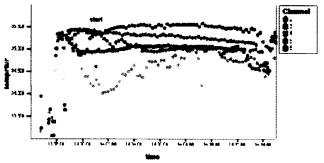
We have tested the catheter (prototype 1) in our unique model of atherosclerosis in cholesterol fed dogs. These dogs are genetically susceptible to cholesterol and generate significant lesions in certain locations such as femoral arteries, however, they do not show any significant lesions in carotid arteries. We therefore introduced the catheter into the femoral and carotid arteries in 5 dogs in order to test the catheter. The catheter was quite safe with no complication, tested in 5 atherosclerotic canine peripheral and coronary arteries in vivo. The catheter detected significant temperature heterogeneity in areas with lesions.







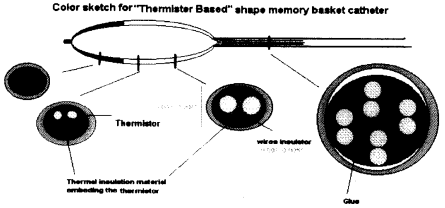
Temperature of Dog Carotid Artery - without lesion



Temperature of Dog Femoral Artery - with lesion

PROTOTYPE II

In this model we have further improved our design and replaced the thermocouple sensors to thermistor type sensor which are more stable and accurate comparing to thermocouple. The following is the schematic design.



Schematic Design

CONCLUSION

- Temperature of arterial wall can be monitored with continuous blood flow using our basket type thermosensor catheter.
- Temperature heterogeneity of atherosclerotic plaques can be seen in vivo.
- The catheter (prototype I) did not cause any damage and had no complications in our animal studies
- Further study to test the prototype II in animal model and then clinical study is considered.

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pH Heterogeneity of Human and Rabbit Atherosclerotic Plaques

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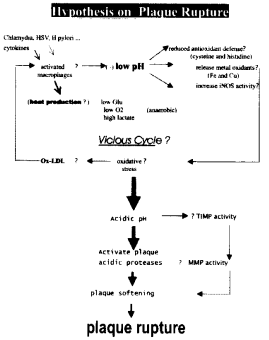
BACKGROUND

- Atherosclerotic plaques have been long shown to be hypoxic, more likely due to increased oxygen consumption by foam cells (ref) and to some degree due to decreased oxygen transmission through the plaque cap (ref). Both lead to an increase in local anaerobic glycolysis.
- It is known that glucose is major fuel of inflammatory cells which rapidly burns during activation of these cells mainly via glycolysis pathway. It explains massive lactate efflux of active macrophages and their extracellular acidification. The turnover rate for the total ATP content of the macrophage in culture is about 10 times per minute: the value for the perfused isolated maximally working rat heart is 22!(ref)
- Monocyte-derived macrophages have been shown to substantially reduce their environmental pH (as low as 5.5), when incubated with Ox-LDL (ref). Macrophages possess both neutral and acidic protease activities toward low density lipoproteins. (ref) Acidic environment may further increase oxidation of LDL (ref).
- Others have shown macrophages in atherosclerotic plaques contain abundant acidic elastolytic enzymes such as catepsins K and S. (ref) Hypoxia and acidic environment increase the metastatic ability (digestion of extracellular matrix and invasion) of cancer cells mainly due to increase activity of cathepsins.(ref)- In addition to degrading elastin, these enzymes can also partially activate matrix metalloprotease enzymes (MMPs). (ref) These data suggest that human macrophages mobilize cysteine proteinases to arm themselves with a powerful effector mechanism that can participate in the pathophysiologic remodeling of the extracellular matrix.
- Plaques vulnerable to rupture are characterized by increased macrophage infiltration and subsequent extracellular matrix digestion
- We have previously shown that atherosclerotic vulnerable plaques are heterogeneous with regard to temperature and that areas at risk of rupture with extensive macrophage infiltration give up more heat.

Hypothesis:

Here we hypothesize atherosclerotic plaques may vary in pH, and that the variation might be useful in predicting progression / instability of the plaque.



METHODS

Fresh human carotid endarterectomy specimens (plaques) were collected from the OR in a biological medium (Dulbeco's Modified Eagles Media) and transferred into a CO2 Water Jacketed Incubator (Forma Scientific) maintained at 37 degrees. The transfer time from the OR to the incubator took approximately 3 minutes. The specimen was removed from the Forma Scientific incubator after 30 minutes and placed in an Isolette Infant Incubator Model C300. The temperature in the incubator was maintained at 37 degrees and humidity between 90 and 100%. The surface of the plaque was wiped clean with absorbable tissue paper. The plaque was mapped out based on 4 gross morphological areas; yellow (lipid rich), calcified, thrombosed and pale (fibrotic degenerative tissue). pH of approximately 25 random points on the plaque using a MI-407P glass tipped needle electrode (Microelectrodes, Inc) and MI-401F micro-reference electrode (Microelectrodes, Inc) mounted on a micromanipulator were measured with a PrepHecT pH meter, Model 370 (Orion). The pH meter was tested and the electrodes were manually calibrated using 2 buffer solutions (pH 4 and pH 7) before each experiment. Temperature at the same point was measured using Model HH23 Microprocessor Thermometer -Type K Thermocouple (Omega). The same method was followed to measure pH and temperature in Watanabe rabbit aorta and human umbilical artery.

To confirm and compare the pH measurements using the microelectrodes, Fluoresence Ratio Imaging Microscopy (FRIM) was applied. A standard pH curve using lyophilized polyacrylamide gel soaked in predetermined pH solutions was made. Human carotid plaques were frozen instantly in the OR using dry ice and sections measuring 15 microns thick were made. The sections were then placed in a pH sensitive probe (BCECF) for 10 seconds and dual emission FRIM was applied using wavelengths 440nm and 490nm. The ratioed image was captured using LSR Concord Digital Image Capture System. The standard curve was applied to the image obtained.

Table. 1 Different Methods for Monitoring pH in Biomedical Applications

	pH Accuracy	pH Resolution	Spatial* Resolution	Non-destructive	Non-invasive	Exits in vivo	Simple*	Cost-effective	Catheter
Electrode	+++	+++	++	-	-	-	+++	+++	-
	++	++	+++	-	-	-	+	++	-
	++	++	+++	+++	-	-	+	++	+++
	+++	+++	-	+++	+++	+++	-	+	+++
	++	+++	+++	+++	-	++	+	++	+++

* *With respect to the size of atherosclerotic plaque < 1.0x1.0x0.5 cm3*

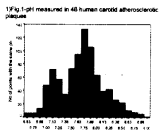
FRIM: Fluorescence Ratio Imaging Microscopy

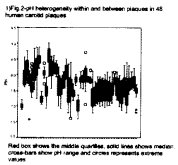
UVS: Ultra-Violet Spectroscopy

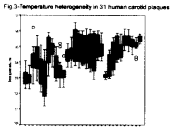
NMRS: Nuclear Magnetic Resonance Spectroscopy

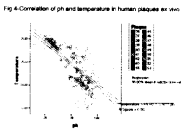
NIRS: Near Infrared Spectroscopy

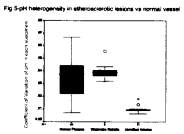
RESULTS

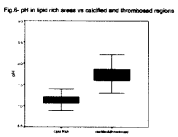


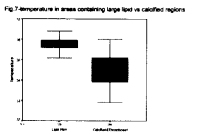












- pH measured in 832 points of 48 CEA was 7.60±..44, in 275 points of 10 WHR was 7.40±..43, and in 233 points of 11 HUA was 7.24±.1. Heterogeneity of pH in CEA, WHR, and HUA were .038±.016, .039±.01, and .009±.0011 respectively (p=.001).
- In CEA, pH of yellow areas containing large lipid cores, were significantly lower than pH in calcified and thrombosed areas (7.27±.59 vs 7.81±.42, p<.000).
- FRIM confirmed microscopic pH heterogeneity in both humans and rabbits.
- pH of CEA was inversely correlated with temperature (r=0.7, p<.000)

LIMITATIONS

The bicarbonate buffer of the biological media (DMEM), in which the specimens were kept after the surgery could alter the absolute values of pH. However, we have tested several other methods including un-washed, washed with normal saline and phosphate buffer, and they did not significantly differ in terms of variation.

Variations in the calcium, water content, blood contamination and thrombosis inside the disrupted specimens may have introduced some variations in both temperature and pH readings. However since the method was applied to both diseased and normal tissues, it cannot explain the difference between them.

Overall, due to several intervening factors, absolute value of pH measured ex vivo may not represent in vivo situations. However, the variations observed in the pH within and between plaques might be more meaningful.

CONCLUSIONS

- Living atherosclerotic plaques exhibit marked pH heterogeneity with lower ph in areas with large lipid core and higher pH in calcified regions.
- pH and temperature of plaque are inversely correlated and are expected to complement each other's value in locating vulnerable plaques
- Correlation between underlying microscopic characteristics of vulnerable plaque and pH measurements remain to be addressed
- The source of pH heterogeneity, its impact on stability of plaques particularly through acidic matrix-degrading enzymes, and its potential clinical utility in locating vulnerable plaques remain to be determined.
- An infrared spectroscopic catheter is being developed to characterize pH, oxygen, and other physiopathologic markers of vulnerability in vivo. Using intravascular magnetic resonance coil catheter for spectroscopic study of plaque may also provide a promising tool to monitor plaque pH. We hope new advancements in magnetic resonance spectroscopy (31P, H+, and lactate) would provide us with a non-invasive tool to detect pH heterogeneity of atherosclerotic plaque.

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Heat Down-Regulates Tumor Necrosis Factor-α and IL-6 in Human Atherosclerotic Plaques

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ABSTRACT

We recently observed that in living human carotid endarterectomy(CEA) specimens, plaques that are warmer than others(by1-3°C) undergo cooling after exposure to heating (40°C-42°C X 15 min), suggesting that heat has an anti-inflammatory action. Therefore, we asked whether heat might regulate tumor necrosis factor-α and IL-6, pro-inflammatory cytokines that promote macrophage proliferation and survival and endothelial cell dysfunction, thrombosis and apoptosis. Seven living, freshly harvested human CEA specimens were divided into two parts, one piece of which was placed at 37°C for 6.3 hrs and other at 42°C for 15 min, followed by six hrs at 37°C, in Dulbecco's modified eagles' medium in a 5% CO_2 incubator. The specimens were fixed, processed and immunostained, using antibodies to TNF-α, IL-6, α-actin and HAM-56. Visual inspection and quantitative morphometry showed a marked decrease in TNF-α immunoreactivity of plaque α-actin positive cells and macrophages (mean 4.42± 2.33SD at 37°C Vs 1.5 + 2.1 at 42°C, P=0.04). Most of the TNF-α immunoreactivity was found in the α-actin positive cells and the reduction of TNF-α by heating was seen primarily in those cells. Thus heating at 40°C-42°C decreases TNF-α and IL-6 immunoreactivity in freshly harvested human carotid plaque. We hypothesized that heat may function to down-regulate macrophage pro-inflammatory activity. The possible therapeutic potential of gentle, non-lethal heating of plaque is being investigated.

INTRODUCTION

The cytokines are multipotent mediators of inflammation and immunity that can affect key functions of vascular wall cells. Growing evidence suggests that cytokines participate as autocrine or paracrine mediators in atherogenesis, as cells in lesions can both produce and respond to these mediators. The functions of vascular wall cells regulated by cytokines may influence lesion initiation, progression, or complication. Cytokines such as tumor necrosis factor-alpha(TNF-α) can regulate the production of monocyte chemoattractant protein-1(MCP-1), a potential signal for directed migration of monocytes into the intima. Cytokines can also regulate genes that encode other growth factors and cytokines themselves. TNF-α can induce IL-1 mRNA in human endothelial and smooth muscle cells. TNF-α can augment the production by vascular cells of macrophage-colony stimulating factor, which may promote growth and activation of mononuclear phagocytes.

Thus, cytokines can influence multiple aspects of atherogenesis and provide new and interesting target for therapeutic intervention.

HYPOTHESIS

In our earlier study we found that in living human carotid endarterectomy specimens, plaques that are warmer than others undergo cooling after exposure to heating, suggesting that heat reduces inflammation.

Considering the above-mentioned facts, we hypothesized that heat may function to down-regulate macrophage pro-inflammatory activity and reduce the production of TNF-α and IL-6.

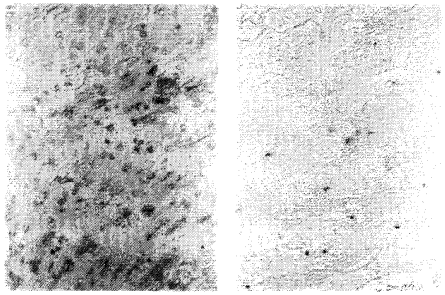
1 2

Picture-1 shows TNF-α positive area and picture-2 shows α-actin positive area on same tissue slide.

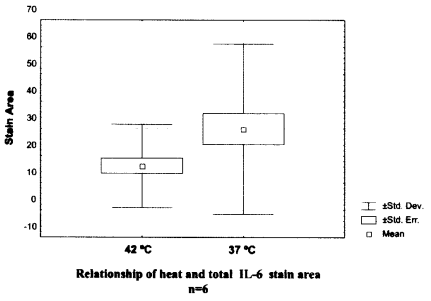
3 4

Picture-3 shows TNF-α positive area and picture-4 shows HAM-56 positive area on same tissue slide.

A B

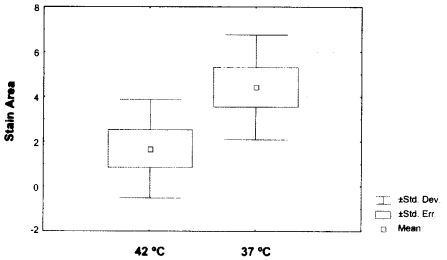


Panel-A shows IL-6 positive areas in an unheated atherosclerotic plaque section. Panel-B depicts IL-6 positive areas in a heated atherosclerotic plaque section



Relationship of heat and total IL-6 stain area
n=6

(Mean 29.80±31.33SD at 37°C Vs 16.25±15.31SD at 42°C, P=0.03)



Relationship of heat and total TNF-α stain area
n = 7

(Mean 4.42±2.33SD at 37°C Vs 1.5±2.1 at 42°C, P=0.04)

CONCLUSION

We conclude that gentle heating down-regulates tumor necrosis factor-alpha and interleukin-6 in human atherosclerotic plaque.

Future Prospects

Currently we are developing a catheter-based heat delivery system, which will allow us to determine whether down-regulation of different cytokines can reduce the burden of inflammation in the plaque, and subsequently stabilize the vulnerable plaque.

Non-invasive Ultrasound Diathermy to Heat Stents and Angioplasty Balloons

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BACKGROUND

- Restenosis occurs in 40-60 % of cases after angioplasty. Infiltration of inflammatory cells and proliferation of smooth muscle cells (SMC) and neointimal formation are major characteristics of restenosis
- Stents reduce the rate of restenosis but in-stent restenosis remains a significant clinical problem.
- Anti-proliferative and apoptotic effects of hyperthermia have long been known, particularly in cancer treatment. (1,2)
- We have previously shown that gentle heating reduces inflammation in atherosclerotic plaques by suppressing pro-inflammatory cytokines and promoting apoptosis of macrophages. (3)
- Schwartz et al (4) has recently identified the inhibitory effect of low level heating on SMC proliferation after balloon injury. The possible non-thermal anti-restenosis effect of ultrasound energy has been raised by Rosenschein et al. (5)
- Thermal preconditioning before rat arterial balloon injury has shown to significantly limit the injury and sustain reduction of intimal thickening. (6)
- Ultrasound (US) hyperthermia is widely used in cancer therapy and in physical therapy where it is known as Diathermy (7). US waves heat subjects based on their absorption and reflection rate. The latter explains the excessive heating effect of US on the surface of bone.

HYPOTHESIS

We hypothesize that ultrasonic heating of stents and angioplasty balloons varies with the type of material.

METHODS

We have used an ultrasound diathermy machine (Chattanoga Inc) as a source of therapeutic ultrasound. This device has a 5-cm2 transducer, which delivers ultrasound energy of 0-2.5-watts/ cm-square and two frequencies: 1 MHz and 3 MHz.

To monitor temperature we employed a 12-channel, 0.001 oC resolution thermometer (Black-Stack, Hartscientific Inc).

In our phantom, we used steak, ground meat, and ribs to test different properties and effects of transmission of ultrasound waves.

We set the phantom with and without container. We used four different sizes of containers, which facilitated the adjustment of biocompatible materials to variable distances in depth from the source beam as well as evaluation of temperature changes in various sites around the material.

Seven different classes of biocompatible materials were used to test their thermal behavior when irradiated by ultrasound waves. These materials were filled with saline solution in some experiments.

The biocompatible materials were:

1 - Silicone: Bio-Sil tubing (Sil-Med Corporation.)

2- PVC: DEHP-plasticized PVC. (LifeShield-Abbott.)

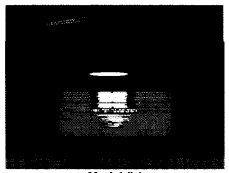
3- PTFE: Polytetrafluoroethylene with carbon covering inside.

4- PTFE: Polytetrafluoroethylene simple.

5- Heat shrinkable tube3M.

6- SciMed angioplasty PTA catheter 8 F. (polyurethane)

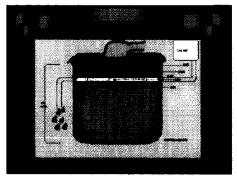
7- Cordis angioplasty PTCA catheter 5 F. (polyurethane)



Model # 1

General Conventions:
- ~ E#1 below the stent-shaped tube (distance varies by model)
- ~ E#2 inside the stent-shaped tube. (Measures media inside the stent).
- ~ E#3 outer surface of stent-shape tube in contact with surrounding tissue.
- ~ E#4 was place in the middle of the distance between the stent-shaped tube and the surface.
- ~ E#5 surface temperature.

This model of a phantom was designed in a container of 4.5-cm depth and filled with beef slices of 0.5 cm. Five electrodes were placed 0.5 cm apart starting from the surface. Stent-shaped tubes of different biocompatible materials filled with saline solution or air were tested at 1 cm from the beam source. Phantom was tested at room temperature.



Model # 2

This is a model of a phantom that shows a container of 19-cm depth, which was filled with beef meat to simulate muscle tissue. Beef slices of 1 cm were carefully and homogeneously cut. Five electrodes shown in the drawing were placed 1 cm apart starting from the surface. Stent-shaped tubes of different biocompatible materials were fixed at 2 cm from the source of ultrasound and filled with saline solution. The complete phantom was put inside a waterbath to reach and maintain 37oC.

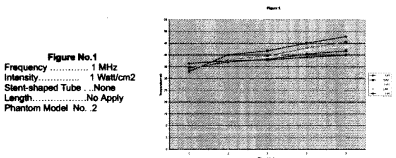
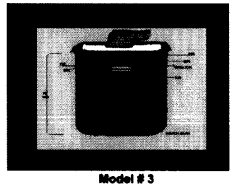


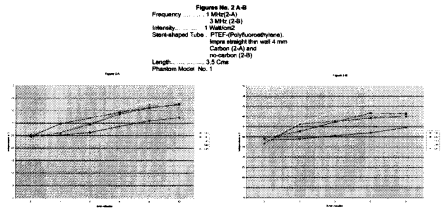
Figure #1. Control situation.

Electrodes were placed 1cm apart as shown in model #2. Normal behavior of temperature distribution inside the phantom without any stent shape tube. It follows the formula: Attenuation (dB) = ½ x Frequency (MHz) x path length (cm) which define the pattern by their distance.



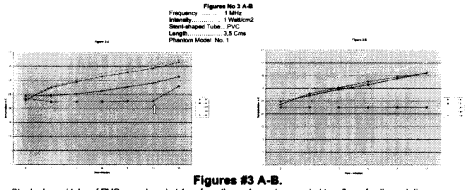
Model # 3

This model of phantom was placed in a container of 19-cm depth and filled with beef slices of 1 cm. Five electrodes were placed 1 cm apart starting from the surface. Two different kinds of balloons were fixed at 2 cm from the surface and the beam source. Balloons were filled with saline solution and tested to simulate non-invasive heating of atherosclerotic plaque during angioplasty. The phantom was placed inside the waterbath at 37oC.



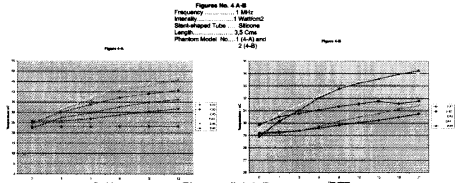
Figures#2 A-B.

The stent -shaped tube was placed at 1cm from the probe simulating high penetration and low attenuation. Notice the slope (in yellow) that corresponds to electrode #3 (surrounding tissue of the stent- shaped PTFE) that reaches high temperature compared to others regardless of the distance from the probe and using two different frequencies.



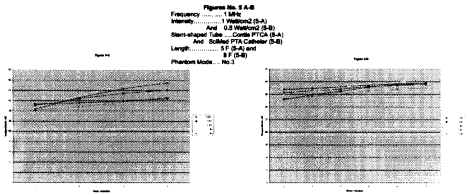
Figures #3 A-B.

Stent -shaped tube of PVC was placed at 1cm from the probe and connected to a flow of saline solution (constant temperature of 23o Celsius). The slope (in yellow) shows how Electrode #3 (surrounding tissue of PVC) heats up regardless of the distance from the surface. Notice constant temperature of Electrode #2 showing the washing effect due to the flow of saline. In figure 3-A the white arrow shows an increment in temperature when flow of saline was stopped (time-minutes 12).



Figures # 4 A-B.

A stent- shaped tube of Silicone was used in these experiments with different models. Silicone was placed at 1 cm from the source of energy in figure 4-A and 2 cm in 4-B. Silicone was filled with air inside in figure 4-A and filled with saline solution in 4-B. Notice the slope (in yellow) that corresponds to electrode #3 (surrounding tissue of stent -shaped tube) reaching high temperature compared with others regardless of the distance from the surface.
Note. Electrode #6 was placed under the container in figure 4-A.



Figures #5 A-B

Balloons were placed at 2 cm from the surface (see phantom model #3). Please notice electrode #3 (surrounding tissue closer to balloon) that heats up compare with others regardless of the distance from the source of energy.
This is an example of non-invasively heating of atherosclerotic plaque during angioplasty.

Picture 1A- B: Ultrasound heating of tissue adjacent to a stent. As shown a fresh piece of uncooked beef is subjected to ultrasound treatment using a CPS system at
1 MHz, 1.5 watts/cm2. The meat is folded over a 7 F wallstent.

Picture 2-This experiment was performed to study the behavior of temperature distribution using real time infrared thermography. As seen in the pictures stent material area reaches 41.9 oC.

LIMITATIONS

- The in vivo environment is hard to simulate; therefore extrapolating these data to the in vivo situation requires caution.
- Unfocused ultrasound may produce excessive heat at the skin level.
- Although focused ultrasound may be the preferred method for uniform, deep heat delivery we have not yet studied focused ultrasound.

We have not studied the materials in an actual stent format (tubular mesh).
Therapeutic ultrasound although clinically approved for other uses, it still warns further caution once applied to cardiovascular system.

RESULTS AND CONCLUSION

We found that according to the acoustic impedance of some biocompatible materials used in our study namely Silicon, PTFE, Polyurethane, and PVC, it is possible to reach an increment in temperature in the tissue surrounding the material when they are irradiated by ultrasound waves in an ex-vivo situation. This is the main reason because our next step involves application of these findings in an in-vivo situation.

When the biocompatible material is placed to 1-cm of distance from the probe, avoiding depth and simulating high intensity focus ultrasound, materials like PVC, Silicon, PTFE, and Polyurethane angioplasty balloons reached the temperature that according to the background information, could shout down inflammatory process responsible for restenosis and inhibit cellular proliferation. Therefore high intensity focus ultrasound (HIFU) is one of the next steps in our study.

A comparative study to examine different methods of diathermy including radiofrequency and microwave is undergoing to address the most preferred methods of heating stents.

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